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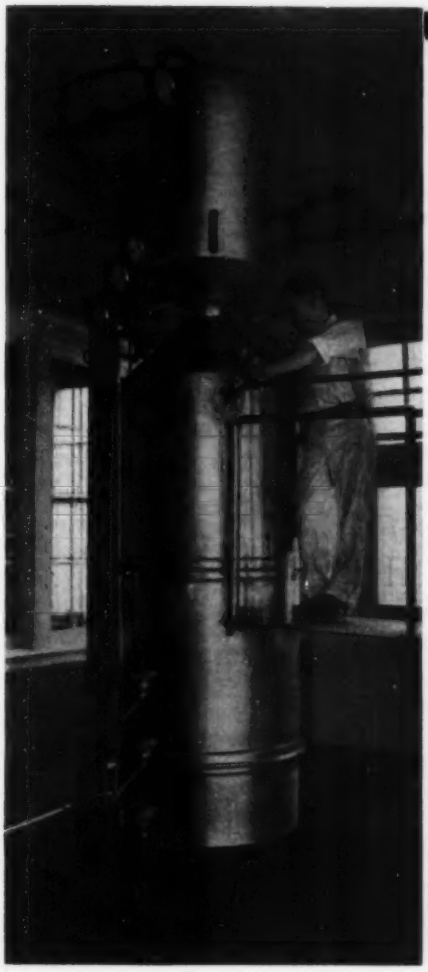

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JOURNAL OF DAIRY SCIENCE

VOLUME XVIII DECEMBER, 1935 NUMBER 12

THE INFLUENCE OF STREPTOCOCCIC INFECTION OF THE UDDER ON THE FLAVOR, CHLORIDE CONTENT, AND BACTERIOLOGICAL QUALITY OF THE MILK PRODUCED*

C. S. BRYAN AND G. M. TROUT

Sections of Bacteriology and Dairy Husbandry, Michigan Agricultural Experiment Station, East Lansing, Michigan

Much attention is being given today to the factors affecting the quality of dairy products. It is recognized that the quality of the raw or basic material plays an important rôle in the general quality of the processed product. For this reason as well as for the assurance of safety from the public health standpoint is the milk supply subjected to inspection.

At the receiving platform, the milk is accepted or rejected generally on its quality as determined by the senses of smell and taste. Other tests made quickly, such as the rapid acid test and the temperature, are sometimes used. During the summer months especially, the methylene blue test is resorted to as a means of segregating poor milk.

Several instances came to our attention where producer's milk had been rejected at the receiving plant, although the milk had been promptly and sufficiently cooled to inhibit bacterial growth. The ordinary bacteriological tests to determine the quality of milk, as well as the flavor and odor along with temperature upon arrival, were used as the basis to accept or reject the milk. On checking the methods of production no explanation could be found for the poor quality of milk, but in these cases streptococci of mastitis were always isolated from the producer's supply. When the infected cows were located and their milk withheld, the producer's supply was again accepted at the plant and the quality returned to normal. Also, immediately cooled, sanitarily-produced fresh milk has shown "off-flavors" which were not of feed origin, but were inherent in the milk. Furthermore, in scoring milk from individual cows or separate herds, one frequently encounters a salty flavor which is entirely foreign to normal cow's milk. Formerly such a flavor in the milk was attributed largely to advanced lactation, but lately the salty flavor has beeen thought to be likewise associated with mastitis. In fact, the salty flavor in milk now arouses suspicion in the

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minds of some milk judges that the milk under examination is from a mastitis-infected cow. In view of these prevailing associations between salty milk, quality of milk, and mastitis infection, an investigation on the subject seemed desirable.

METHODS

Samples of milk were obtained from three herds, two of which were known to have mastitis while the third was entirely mastitis-free. The udders of the cows were wiped with a moist cloth, several streams of milk from each quarter were discharged, and the samples collected by milking directly into the sterile containers. The samples were iced immediately so that off flavors due to bacterial growth would not develop.

Upon arrival at the laboratory the methylene blue reduction time and standard plate count were determined on all samples. In addition Breed's smears were prepared and a leucocyte or cell count made and the percentage of chlorides present was determined according to the method of Rosell (6). Cultural examinations, using a differential medium, were made of all samples for the presence of streptococci of mastitis (1). Streptococcic mastitis infection in the cows was established by the presence of the streptococci in the milk and indurations of the udder tissue. Future use of the term mastitis refers to streptococcic mastitis. The samples were then scored organoleptically for flavor and odor, the samples being keyed to prevent during scoring identity of their source and, therefore, possible correlation of flavor. In all 258 samples from 60 cows covering a period of six weeks were studied.

RESULTS

Effect of streptococcic infection upon the chloride content and flavor of milk.

In preliminary studies some samples of milk had been taken at random from 26 individual cows of a herd which was known to be infected with streptococcic mastitis. The samples were tested organoleptically for salt flavor only. The samples were then identified and the presence or absence of udder infection was recorded.

Eight of the cows from which samples were taken were infected. Of these samples five were definitely salty. Saltiness was not noted in any of the samples from the non-infected animals. All of the cows were yielding a good flow of milk, which may account for the fact that a salty condition was not noted in any of the samples from the non-infected cows. The quantity of the milk yield as well as the extent of infection may have accounted for the fact that saltiness was not noted in some of the samples from the infected cows, although it was noted in the majority of them.

Following this introductory work, studies were made on samples of milk from individual cows of two infected herds and from one non-infected herd

to determine the percentage of chloride present and also to ascertain the flavor quality with special reference to salty flavor. The data in Table 1 represent a six-weeks' study of the milk for chlorides from Herd A, composed of Holstein cows of which 40 per cent showed streptococcic infection.

TABLE 1

A comparison of the chloride content of milk from non-infected and that from infected cows

HERD A

DATE	NON-INFECTED COWS (11–12)				INFECTED COWS (8)			
	No. of samples showing per cent Cl. between			Average Cl. content (%)	No. of samples showing per cent Cl. between			Average Cl. content (%)
	0–.14	.14–.16	.16+		0–.14	.14–.16	.16+	
3/27	0	4	8	.180	0	0	8	.206
4/3	1	5	5	.162	0	4	4	.173
4/10	3	5	3	.151	2	2	4	.171
4/17	0	3	8	.179	0	3	5	.186
4/24	0	7	4	.164	0	3	5	.214
5/1	0	5	6	.168	0	1	7	.204
Total	4	29	34	.....	2	13	33	.....
Per cent total	6	43	51	.167	3	27	70	.192

Seventy per cent of the individual samples of milk from the infected cows had above the normal limit of 0.16 per cent chloride present, with an average of 0.192 per cent over all samples. The daily chloride content showed a range from an average of 0.171 to 0.206 per cent. Although the average chloride content of the milk from the non-infected cows was considerably lower than that from the infected cows, being 0.167 per cent, it would seem that this was high in light of the figures given for the chloride of normal milk by Hucker (3). Fifty-one per cent of the samples from non-infected cows showed greater than 0.16 per cent chloride, with a daily range from 0.151 to 0.18 per cent.

Similar studies were made on Herd B consisting of Holsteins of which the extent of infection was not so great. The data obtained are presented in Table 2.

The infected samples from this herd were found to have considerably more than 0.16 per cent chlorides. The average was 0.2263 per cent and the daily range was 0.17 to 0.28 per cent. This is much greater than that found in Herd A. The non-infected samples also showed on the average more chlorides present than the non-infected samples from Herd A. Although 53 per cent of the samples from the non-infected cows had less than 0.16 per cent chlorides, while 43 per cent had more than 0.16 per cent,

TABLE 2

A comparison of the chloride content of milk from non-infected and that from infected cows

HERD B

DATE	NON-INFECTED COWS (11–13)				INFECTED COWS (1–3)			
	No. of samples showing per cent Cl. between			Average Cl. content (%)	No. of samples showing per cent Cl. between			Average Cl. content (%)
	0 – .14	.14 – .16	.16 +		0 – .14	.14 – .16	.16 +	
3/27	0	5	6	.1691	0	0	1	.23
3/29	0	3	8	.1809	0	0	1	.27
4/3	0	7	4	.1641	0	0	1	.27
4/5	2	2	9	.1700	0	0	1	.20
4/10	1	6	6	.1669	0	0	1	.17
4/17	0	6	7	.1861	0	0	1	.23
4/19	1	8	4	.1746	0	0	1	.19
4/24	0	10	1	.1533	0	0	1	.28
5/1	0	10	1	.1563	0	0	3	.2166
Total	4	57	46	.....	0	0	11	.....
Per cent total	3.7	53.3	43.0	.1694	0	0	100	.2263

the average from all samples was 0.1694 per cent with a daily range during the six weeks' period from a low of 0.1533 to a high of 0.1861 per cent.

Chloride determinations were made also of samples of milk from individual cows from a Guernsey herd negative to mastitis for 36 months. These data are presented in Table 3.

The average chloride content of the 35 samples was 0.1374, with a range from a low of 0.11 to a high of 0.19 per cent. Of the 35 samples only three had greater than 0.16 per cent chloride, being 0.17, 0.18, and 0.19 per cent. The cows from which these samples were taken were practically dry. A comparison of data in Tables 1, 2 and 3 shows a materially lower per cent of chlorides in the milk from a non-infected herd than that from non-infected cows of an infected herd.

In view of these data, the flavor and flavor scores of the same milk as was used in determining the chloride content is of interest. The data obtained from scoring the samples of milk from the various herds are presented in Tables 4, 5, and 3.

Of the infected samples from Herd A, 56 per cent were criticized for being "salty" and of the non-infected samples from the same herd 25 per cent were criticized for "salty" flavor.

Only one cow in Herd B consistently showed streptococcic infection, although two additional cows, recently freshened, were likewise infected. Samples from these infected cows were criticized for "saltiness" in 90.9

TABLE 3

The per cent chlorides, flavor score, and flavor criticism of samples of milk from individual cows of a non-infected herd, negative for 36 months

NO. OF COW	CHLORIDES (%)	CLASS OF MILK*					SCORE	CRITICISM
		Excel.	Good	Fair	Poor	Bad		
1	.12		+				22	———
2	.14	+					23	———
3	.14		+				21	sl. cowy
4	.12	+					23	———
5	.15	+					23	———
6	.13		+				22	———
7	.11		+				22	———
8	.13			+			21	sl. flat
9	.14		+				22	———
10	.12	+					23	———
11	.14			+			20	flat
12	.14		+				22	———
13	.15	+					23	———
14	.14		+				22	———
15	.15			+			19	salty
16	.14		+				22	———
17	.12		+				21	sl. feedy
18	.16			+			20	sl. salty
19	.14		+				22	———
20	.12	+					23	———
21	.13			+			18	salty
22	.14		+				21	sl. feedy
23	.14		+				22	———
24	.12	+					23	———
25	.13	+					23	———
26	.11		+				22	———
27	.17			+			18	salty
28	.12	No samples taken						
29	.11	"	"	"				
30	.15	"	"	"				
31 (dry)	.19	"	"	"				
32	.16	"	"	"				
33 (almost dry)	.18	"	"	"				
34	.14	"	"	"				
35	.12	"	"	"				
Average	0.1374							

*Key: Excellent, score 23–25
Good, " 21–23
Fair, " 18–21
Poor, " 12–18
Bad, " 0–12

per cent of the cases, whereas 20.5 per cent of the non-infected samples and only 14.8 per cent of the samples from the negative herd were similarly criticized.

Not all samples from infected cows were criticized in each case for salty flavor, but the majority of them were as shown by the above figures. Just as the per cent of chlorides present varied so did the intensity of flavor.

Apparently there existed some relationship between the naturally high

TABLE 4

A comparison of the flavor quality of milk from non-infected and that from infected cows

HERD A

DATE	NON-INFECTED COWS (11–12)				INFECTED COWS (8)			
	No. of samples scoring between		Average flavor score*	Number samples criticized "salty"	No. of samples scoring between		Average flavor score*	Number samples criticized as "salty"
	18–20	21–25			18–20	21–25		
3/27	6	6	20.7	5	5	3	19.7	3
4/3	7	4	20.2	3	3	5	20.6	2
4/10	6	5	20.7	3	5	3	20.1	5
4/17	2	9	21.2	2	4	4	20.6	5
4/24	7	4	19.9	4	8	0	19.1	6
5/1	1	10	21.3	0	6	2	19.6	6
Total	29	38	.....	17	31	17	.....	27
Per cent total	43	57	20.67	25	65	35	19.54	56

*Perfect score—25.

chloride content and the increase due to streptococci which affected the flavor, inasmuch as it was sometimes observed that medium high chloride content samples did not always merit a salty flavor criticism. This is shown

TABLE 5

A comparison of the flavor quality of milk from non-infected and that from infected cows

HERD B

DATE	NON-INFECTED COWS (11–13)				INFECTED COWS (1–3)			
	No. of samples scoring between		Average flavor score*	Number samples criticized "salty"	No. of samples scoring between		Average flavor score*	Number samples criticized as "salty" or "sl."
	18–20	21–25			18–20	22–25		
3/27	3	8	21.63	2	1	0	18.0	1
3/29	2	9	21.81	3	1	0	18.0	1
4/3	2	9	21.72	2	1	0	18.0	1
4/5	5	8	20.38	5	1	0	18.0	1
4/10	6	7	20.69	2	1	0	19.0	1
4/17	4	9	20.96	2	1	0	18.0	1
4/19	5	8	20.65	2	1	0	18.0	1
4/24	1	10	21.81	1	1	0	17.0	1
5/1	3	8	21.27	3	3	0	18.3	2
Total	31	76	.....	22	11	0	.....	10
Per cent total	28.97	71.03	21.17	20.5	100	0	18.1	90.90

* Perfect score—25.

TABLE 6

The per cent chlorides, flavor score, and flavor criticism of samples of milk from infected cows at weekly intervals covering a period of six weeks

HERD A

COW NO.	CHLORIDES (%)						SCORE (a)						FLAVOR (b)		
	Date						Date						Date		
	3/27	4/3	4/10	4/17	4/24	5/1	3/27	4/3	4/10	4/17	4/24	5/1	3/27	4/3	4/10
1	.19	.23	.16	.17	.28	.19	23	22	22	21	18	20	–	–	–
2	.26	.23	.26	.31	.29	.22	18	19	20	18	18	18	+++	++	++
4	.20	.15	.14	.16	.18	.17	19	19	21	21	20	20	–	–	+
8	.17	.14	.13	.14	.14	.15	19	21	19	21.5	19	21	–	–	–
16	.17	.18	.13	.18	.19	.17	22	23	21	21.5	20	22	–	–	+
17	.20	.15	.19	.19	.16	.19	19	22	20	21.5	19	19	++	–	–
21	.19	.12	.18	.19	.22	.20	–	21	20	20	20	18.5	–	–	++
25	.27	.18	.18	.15	.25	.29	18	18	18	20	19	18	+++	+++	+++
Herd B															
10	.23	.27	.17	.23	.28	.22	18	18	19	18	17	18	+++	+++	+++

Key to Table 6.

[a] Perfect score, 25.

[b] Not noticeably salty, although the flavor may have been off.

\+ sl. salty.

++ salty.

+++ very salty.

in Table 6 where the per cent chlorides, numerical flavor score, and flavor criticism are respectively given of weekly samples of milk from infected cows.

The daily variation in chloride content and flavor of infected samples taken weekly over a period of six weeks is presented in Table 6.

It seems from the data presented in Table 6 that when milk from an infected cow showed a consistently high chloride content that the milk was consistently criticized for being salty, or very salty. On the other hand, when there existed a fluctuation and wide variation in per cent chlorides the flavor was not consistently salty, and furthermore, the salty flavor criticism did not always coincide with the per cent chloride present.

Effect of streptococcic infection upon bacteriological quality of milk.

At the same time that the milk samples were collected for organoleptic examination and determination of the chloride content, cow composite and individual quarter samples were collected for bacteriological examination. The methylene blue reduction test was run, bacteria count determined, and a leucocyte count made; also each sample was cultured for streptococci. These results are classified according to the recognized standards or counts.

The results from examination of the non-infected milk samples are given to permit a comparison of the infected and non-infected samples. These data are presented in Table 7.

The number of non-infected cows in Herds A and B are indicated in the Table. It should be kept in mind that these are non-infected cows present in infected herds. In Herd A all except one of the non-infected samples were of class one methylene blue milk. This one was class two at the time of the first examination. The cow concerned was near the end of her lactation period. Two of the samples from Herd B were of class two at the time of the first examination, following which all were consistently class one. All samples from Herd C were class one; this herd had been mastitis-free for a period of 36 months. The total number of samples examined is given in Table 7, as well as those falling in the various classes according to the tests employed. On the basis of the methylene blue test 98.5 per cent of all samples were class one and the remaining one and one-half per cent were class two. No class three or four milks were obtained from non-infected cows.

These samples were further classified according to the numbers of leucocytes per cubic centimeter. In a study of the relation of streptococci infection to udder induration Hucker and Udall (4) established the normal value of 500,000 or less per cubic centimeter. Other workers (5) (2) have set the normal value at 1,000,000 or less per cubic centimeter. Recognizing the value of these standards, the milk samples were classified on this basis.

All of the samples from Herd A contained fewer than 1,000,000 leucocytes per cubic centimeter; of these only six contained more than 500,000 at any examination during the six weeks' period of this study. One sample

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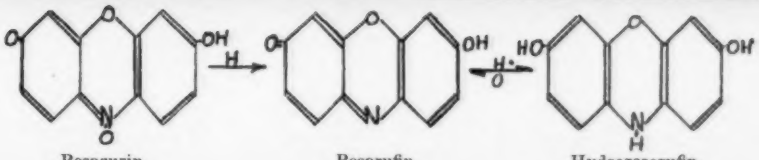

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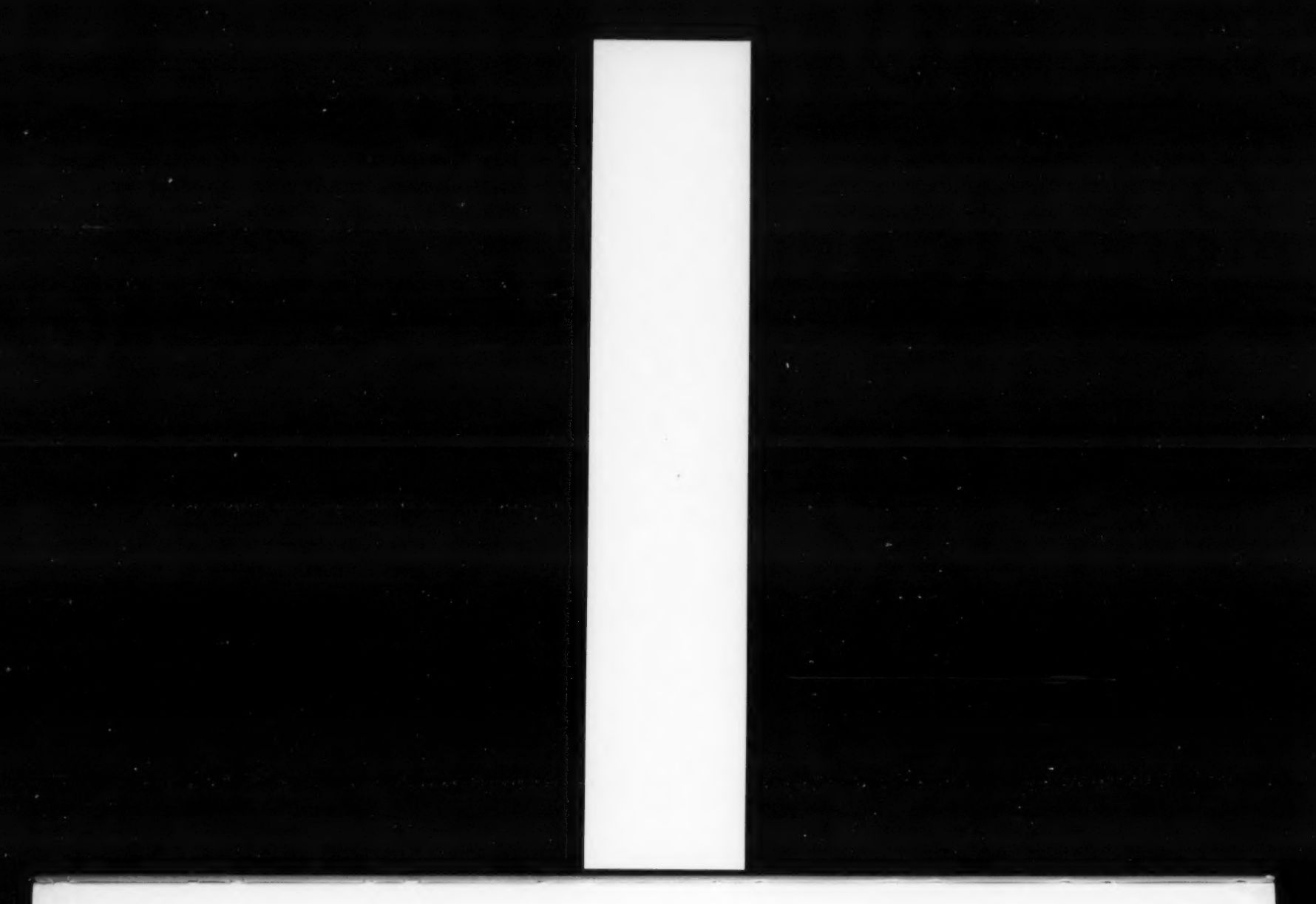

TABLE 7

Composite milk samples of non-infected cows present in streptococcus infected herds

	DATE	NO. OF COWS	METH. BLUE CLASS				LEUCOCYTES PER CC.			BACTERIA PER CC.				
			1	2	3	4	less than 500,000	500,000 to 1,000,000	more than 1,000,000	less than 100	100 to 200	200 to 500	500 to 1000	more than 1000
Herd A	3/27	12	11	1	0	0	9	3	0	3	0	3	4	2
	4/3	11*	11	0	0	0	9	2	0	1	2	2	5	1
	4/10	11	11	0	0	0	10	1	0	0	1	2	7	1
	4/17	11	11	0	0	0	11	0	0	0	0	4	6	1
	4/24	11	11	0	0	0	11	0	0	0	0	7	4	0
	5/1	11	11	0	0	0	11	0	0	0	0	4	7	0
							*1 cow ended lactation period							
Herd B	3/27	11	9	2	0	0	10	1	0	0	2	2	7	0
	3/29	11	11	0	0	0	11	0	0	0	2	1	3	5
	4/3	11	11	0	0	0	11	0	0	1	0	1	8	1
	4/5	13*	13	0	0	0	12	0	1	0	0	3	10	0
	4/10	13	13	0	0	0	13	0	0	0	0	4	9	0
	4/17	13	13	0	0	0	13	0	0	1	0	4	7	1
	4/19	13	13	0	0	0	12	1	0	0	0	5	8	0
	4/24	11**	11	0	0	0	11	0	0	0	1	5	5	0
	5/1	11	11	0	0	0	10	1	0	0	1	5	5	0
							*2 cows freshened			**2 cows ended lactation period				
Herd C (mastitis-free)		35	35	0	0	0	35	0	0	25	8	3	0	0
	Total	209	206	3	0	0	199	9	1	30	16	55	96	12
	Per cent of samples		98.5	1.5	0	0	95	4.3	0.7	14.4	7.7	26.3	45.8	5.8

from Herd B contained leucocytes in excess of 1,000,000 per cubic centimeter and three samples had counts in excess of 500,000 but less than 1,000,000 at any examination. The remaining milks from Herds A and B, and all samples from Herd C contained less than 500,000 leucocytes per cubic centimeter. The distribution of the samples, on the basis of leucocyte content, indicates that counts of over 1,000,000 per cubic centimeter were not very frequent in the milk from the non-infected cows. In fact the tendency was for the leucocyte content to be much lower than 1,000,000, as evidenced by 95 per cent of the samples falling in the class with less than 500,000 leucocytes, 4.3 per cent in the class from 500,000 to 1,000,000 and only 0.7 per cent of the samples containing leucocytes in excess of 1,000,000 per cubic centimeter.

The bacteria count of the samples varied from one sampling to the next. As indicated in Table 7, approximately 50 per cent of all samples from the non-infected cows contained less than 500 bacteria per cubic centimeter. In addition 45.8 per cent of the remaining milk samples contained between 500 to 1,000, and only 5.8 per cent over 1,000 bacteria per cubic centimeter.

The values obtained upon examination of milk from the non-infected cows present some basis of comparison for the milk from the streptococcus-infected cows. The milk from the infected cows in Herds A and B was studied; these results are summarized in Table 8.

A total of 57 streptococcus-infected milk samples were studied. Upon comparing the percentage of infected samples occurring in the various classes, it is noted that 52.6 per cent of the samples were class one methylene blue as compared to 98.5 per cent of the non-infected samples. A definite decrease in the quality of streptococcus-infected milk is evident, as determined by the methylene blue test, since 28 per cent were class two, 15.9 per cent class three, and 3.5 per cent class four as compared to the 1.5 per cent of non-infected samples lower than class one.

Similarly, the leucocyte content of the infected milk was increased. Fifty-three and seven-tenths per cent of the samples contained over 1,000,000 leucocytes per cubic centimeter and 20 per cent had counts above 500,000 but lower than 1,000,000 with only 26.3 below 500,000 per cubic centimeter. The increase in leucocytes is rather marked when these values are compared with those from the non-infected samples (Table 7).

The bacteria count of the milk from the individual cows varied from day to day, as evidenced by the shift in counts of many of the samples. Of the infected samples 7 per cent had counts between 200 and 500, 24.5 per cent from 500 to 1,000, and 68.5 per cent contained more than 1,000 bacteria per cubic centimeter. Only 5.8 per cent of the non-infected samples had bacteria counts of more than 1,000 per cubic centimeter. It is evident that streptococcus infection of the udder caused a marked increase in the total number of bacteria present in the milk produced.

TABLE 8

Composite milk samples of streptococcus infected cows present in herds A and B

	DATE	NO. OF COWS	METH. BLUE CLASS				LEUCOCYTES PER CC.			BACTERIA PER CC.				
			1	2	3	4	less than 500,000	500,000 to 1,000,000	more than 1,000,000	less than 100	100 to 200	200 to 500	1000 500 to	more than 1000
Herd A	3/27	8	5	2	1	0	2	4	2	0	0	1	1	6
	4/3	8	5	0	1	2	2	0	6	0	0	1	1	6
	4/10	8	7	1	0	0	4	0	4	0	0	0	5	3
	4/17	8	8	0	0	0	2	3	3	0	0	2	2	4
	4/24	8	3	1	4	0	2	2	4	0	0	0	2	6
	5/1	8	2	5	1	0	3	2	3	0	0	0	3	5
Herd B	3/27	1	0	1	0	0	0	0	1	0	0	0	0	1
	3/29	1	0	0	1	0	0	0	1	0	0	0	0	1
	4/3	1	0	0	1	0	0	0	1	0	0	0	0	1
	4/5	1	0	1	0	0	0	0	1	0	0	0	0	1
	4/10	1	0	1	0	0	0	0	1	0	0	0	0	1
	4/17	1	0	1	0	0	0	0	1	0	0	0	0	1
	4/19	1	0	1	0	0	0	0	1	0	0	0	0	1
	4/24	1	0	1	0	0	0	0	1	0	0	0	0	1
	5/1	1	0	1	0	0	0	0	1	0	0	0	0	1
	Total	57	30	16	9	2	15	11	31	0	0	4	14	39
	Per cent of samples		52.6	28	15.9	3.5	26.3	20	53.7	0	0	7	24.5	68.5

TABLE 9

Results of examination of milk from infected and non-infected quarters of the streptococcus infected cows in Herd A

DATE	NO. OF QUARTERS	METH. BLUE CLASS				LEUCOCYTES PER CC.			BACTERIA PER CC.				
		1	2	3	4	less than 500,000	500,000 to 1,000,000	more than 1,000,000	less than 100	100 to 200	200 to 500	500 to 1000	more than 1000
		Examination of milk from the streptococcus infected quarters											
3/27	14	8	1	2	3	1	8	5	0	0	0	3	11
4/3	16	12	1	3	0	4	0	12	1	0	1	5	9
4/10	16	14	1	1	0	4	5	7	2	1	0	4	9
4/17	16	16	0	0	0	5	4	7	0	1	2	1	12
Total ..	62	51	3	6	3	14	17	31	3	2	3	13	41
Per cent of samples		80.8	4.8	9.6	4.8	23	27	50	4.8	3.2	4.8	20.9	66.3
		Examination of milk from the non-infected quarters											
3/27	18	15	3	0	0	9	7	2	2	0	7	4	5
4/3	16	15	0	1	0	8	2	6	2	1	1	4	8
4/10	16	16	0	0	0	11	2	3	4	0	0	4	8
4/17	16	16	0	0	0	8	5	3	0	1	4	3	8
Total ..	66	62	3	1	0	36	16	14	8	2	12	15	29
Per cent of samples		94	4.5	1.5	0	54.5	24.2	21.3	12.1	3	18.1	22.8	56.1

The fact that a cow may have a streptococcus infection in one, two, three or all four of her quarters raised the question concerning the quality of the milk secreted by the various quarters. In order to make this study, individual quarter samples of milk were collected from the infected cows in Herds A and B. The results of examination of these infected and non-infected quarters are summarized in Table 9.

The methylene blue rating of the milk from the infected quarters is lower than that from the non-infected quarters. Only 80.9 per cent of the infected quarters secreted class one milk, as compared to 94 per cent of the non-infected quarters. Ninety-four per cent of the non-infected quarters of infected cows produced class one milk, as compared to 98.5 per cent of non-infected cows producing class one milk. The per cent of class two milk from both infected and non-infected quarters was quite constant, being 4.8 and 4.5. Only 1.5 per cent of the non-infected quarters was class three and none class four, while the infected quarters yielded 9.6 per cent of class three and 4.8 per cent of class four milk.

The leucocyte counts presented somewhat the same picture with approximately the same percentage of infected and non-infected samples ranging between 500,000 and 1,000,000 per cubic centimeter. These values are 27 and 24.2 per cent, respectively. The infected milks showed a decrease in the percentage of samples with less than 500,000 and a like increase in the percentage of samples with leucocyte counts in excess of 1,000,000 per cubic centimeter.

The data indicate that the infected quarters yield milk of higher bacteria count than that of non-infected quarters. Sixty-six per cent of infected quarters secreted milk containing more than 1,000 bacteria per cubic centimeter as compared to 56.1 per cent of the non-infected quarters. These results seem to indicate that the infection in the involved quarters may exert some influence on the milk secreted by the non-infected quarters, since an increased number of streptococcus-free quarters gave a milk of lower quality than the milk produced by mastitis-free cows.

In order to bring out more clearly, the relationship of streptococcus infection of the udder to the quality of milk produced the data are summarized in Table 10. The percentage of cow composite and quarter milk samples from both infected and non-infected cows that fall into the various classes of the tests employed are presented.

These data indicate that the streptococcus-free cows produced milk of the highest quality as determined by the methylene blue test, leucocyte count, and bacteria count, with the milk from the non-infected quarters of infected cows of next highest quality and the milk from infected quarters and streptococcus infected cows of lowest quality.

The producer's milk supply, as it arrived at the receiving plant, was also studied during the time of the investigation. The quality of the whole

TABLE 10

The percentage of milk samples studied that occurred in the various classes of the tests employed

SAMPLES		METH. BLUE CLASS				LEUCOCYTES PER CC.			BACTERIA PER CC.		
		1	2	3	4	less than 500,000	500,000 to 1,000,000	more than 1,000,000	less than 500	500 to 1000	more than 1000
Cow § composite	(Infected	52.6	28	15.9	3.5	26.3	20	53.7	7	24.5	68.5
	Non-(infected	98.5	1.5	0	0	95	4.3	0.7	50*	45.8	5.8
Quarter §	(Infected	80.8	4.8	9.6	4.8	23	27	50	12.8	20.9	66.3
	Non-(infected	94	4.5	1.5	0	54.5	24.2	21.3	33.2	22.8	56.1

§ All figures represent percentage of samples occurring in the classes.

* Of these 14.4 per cent had counts of less than 100 bacteria per cc. of milk.

supply was consistently low during the time that the milk from the streptococcus-infected cows was included. When the milk from the infected cows was withheld the quality of the producer's supply was greatly improved. This is very important when dairymen are desirous of producing a high quality milk.

SUMMARY

Samples of milk were secured from individual cows from three herds, two of which showed streptococcic infection while the third showed no streptococcic infection. These samples were tested, organoleptically for salty flavor and for per cent chlorides. Fifty-six per cent and 90.9 per cent of the infected samples from the two infected herds were criticized for having a salty flavor. Twenty-five per cent and 20.5 per cent of the non-infected samples from the infected herds were found to have a salty flavor, as compared to 14.8 per cent of the samples from the negative herd, these latter being from cows almost dry or at least far advanced in lactation.

The averages of the per cent chlorides in the infected samples were 0.192 and 0.2263 for the two herds studied, with the non-infected samples of the same herds averaging 0.167 and 0.1694 per cent, respectively. The milk from the non-infected herd averaged only 0.1374 per cent chloride.

Milk from the infected cows of the two herds scored averages of 19.54 and 18.1 points while the milk from the non-infected cows of the same herds scored averages of 20.67 and 21.17, respectively. The milk of the non-infected herd averaged a score of 21.59.

Although the flavor of mastitis milk was not always salty, the majority of the samples were so criticized. Factors such as stage of lactation, milk yield, and extent of infection, undoubtedly have a marked influence upon the salty flavor of the milk.

The methylene blue test was run on streptococcus-infected and non-infected cow composite and quarter samples of milk. In addition, the leucocyte and bacteria counts of all samples were determined. The milk from the streptococcus-free cows was of higher quality, as determined by the methylene blue reduction test and the number of leucocytes and bacteria per cubic centimeter. The next highest quality of milk was produced by the non-infected quarters of streptococcus-infected cows.

A streptococcus infection in one, two or three quarters of a cow seemed to influence the quality of milk secreted by the non-infected quarters of the same cow. On this account the milk produced by non-infected cows was of higher quality than that secreted by the non-infected quarters of a streptococcus-infected cow.

The infected cow composite and quarter samples of milk were of lowest quality on the basis of the tests employed. They were of lower methylene blue class milk and contained the highest number of leucocytes and bac-

teria per cubic centimeter of milk. These facts are most important when dairymen are interested in the production of high quality milk.

The elimination from the producer's supply of milk from the streptococcus-infected cows in two herds resulted in an increase in the quality of the producer's milk.

Although most of the milk from streptococcus-infected cows had a salty flavor, high chloride content, high leucocyte and bacteria counts, and was of lower quality as determined by the methylene blue test, none of these determinations can be used alone to make an accurate diagnosis of streptococcic mastitis.

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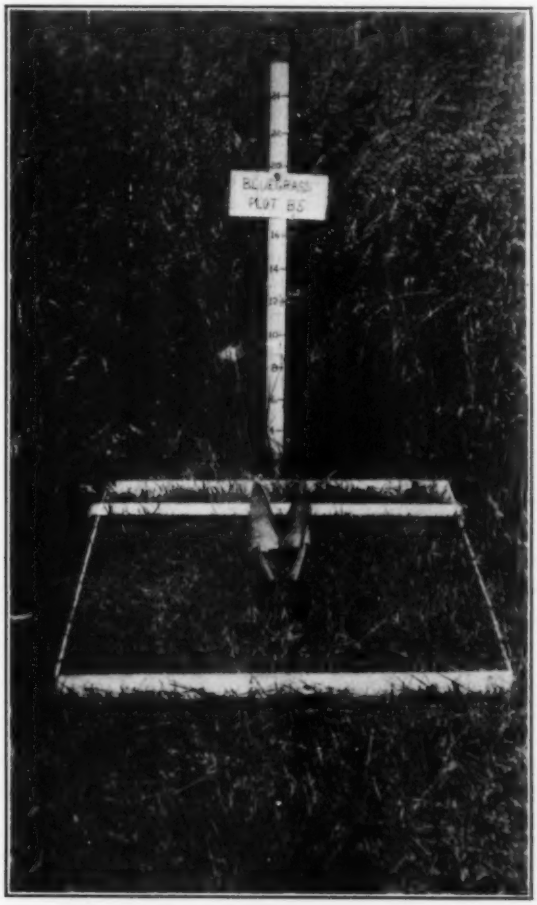
A METHOD OF HARVESTING SAMPLES OF PASTURE FORAGE

W. B. NEVENS AND A. F. KUHLMAN

Department of Dairy Husbandry, University of Illinois, Urbana, Illinois

A simple device for use in the harvesting of small samples of pasture forage was made from strap iron $1\frac{1}{4}$ inches in width and $\frac{3}{16}$ inch in thickness. The iron was shaped into a rectangular frame 20.79 inches by 20.79 inches inside measurement. A separate bar of the same material fits loosely over the frame. The ends of the bar turn downward to keep it in place. (Fig. 1.) The area enclosed by the frame is 3 square feet.



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After selecting the area to be harvested, the frame is held in a horizontal position and dropped into place. When the crop is tall, some of the plants are likely to be bent over by the frame. These may be straightened and those which should be within the frame and those which should be outside may be adjusted to their proper positions by drawing the index and middle fingers along the inside and outside of the frame. A sheep shears is used for cutting the forage. Before the removable bar is put into place, a "swath" is cut by resting the base of the blades on the edge of the frame next to the operator. After the removal of the first swath, the metal bar is laid on the frame and the cutting completed by moving the bar forward as needed, at all times resting the shears on the bar. When the forage is of sufficient length, the harvest may be facilitated by an assistant who grasps a bunch of the forage, holds it during cutting and lifts it out of the way of the next stroke of the shears.

This method accomplishes several purposes: a rigidly-defined area is harvested; the position of the frame does not change during the collection of the sample because of the weight of the device; the forage is cut without scattering the sample, an accomplishment which is difficult when a hand sickle is used; the forage is cut at a uniform height from the ground; the sample is of convenient size for drying.

THE VITAMIN A ACTIVITY OF BUTTER PRODUCED BY COWS FED ALFALFA HAY AND SOYBEAN HAY CUT IN DIFFERENT STAGES OF MATURITY*

J. H. HILTON, S. M. HAUGE AND J. W. WILBUR

Departments of Dairy Husbandry and Research Chemical Laboratory
Purdue University Agricultural Experiment Station, Lafayette, Indiana

The production of butter of high vitamin A value is largely dependent on the vitamin A activity of the feeds fed the cows. The chief sources of vitamin A in the rations of cows are pasture grasses and various types of hay. Hilton, Hauge and Wilbur (1) found that, although the vitamin A activity of butter produced under winter feeding conditions declines when the cows receive hay of low vitamin A value such as timothy hay, it was possible through the feeding of good quality alfalfa to produce butter equal in vitamin A value to that produced when the cows were on pasture. It was also found that soybean hay was less effective in maintaining this high vitamin A activity of butter than was alfalfa hay.

With this close correlation between the vitamin A value of hay fed the cows and the vitamin A value of the butter produced, it is highly essential that hay of high vitamin A activity be fed to dairy cows in order to produce butter of high vitamin A value. However, hays vary considerably in their vitamin A value. This variation may be due not only to differences in the species of the plants from which the hay is produced, but also to the maturity of the plants when harvested and to the conditions of the curing process. Numerous investigators (2–5) have shown that the drying of alfalfa by means of mechanical driers tends to preserve the vitamin A value while the field curing process is more or less destructive to the vitamin A value. Recently, Hauge (6) has shown that the vitamin A value of alfalfa varies with the stage of maturity of the plants, young alfalfa (10–12 inches high) possessing greater vitamin A value than plants in the full bloom stage.

Therefore, it seemed desirable to extend these studies to include a comparison of the vitamin A activity of soybean and alfalfa hay, cut at different stages of maturity and cured by two different processes; and, to study the relationship between the vitamin A activity of the hays and the butters produced by cows fed these hays.

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EXPERIMENTAL

The Hay Samples

The samples of hay used in these experiments were prepared from alfalfa and soybean plants harvested in two different stages of maturity. Portions of each cutting of each hay were field cured and artificially dried. Eight samples of hay were prepared and classified according to the time of harvesting and method of curing.

F.C.Y.A.—Field cured young alfalfa prepared from plants when they were from 10–12 inches high and before the bloom stage.

A.D.Y.A.—Artificially dried young alfalfa hay prepared from plants when they were from 10–12 inches high, before the bloom stage and cured in a mechanical hay drier.

F.C.L.A.—Field cured late alfalfa hay prepared from plants cut at the usual period in the bloom stage.

A.D.L.A.—Artificially dried late alfalfa hay prepared from plants cut at the usual period, in the bloom stage and cured in a mechanical hay drier.

F.C.Y.S.—Field cured young soybean hay prepared from plants 12–15 inches high when the pods were just starting to form on the lower branches.

A.D.Y.S.—Artificially dried young soybean hay prepared from plants 12–15 inches high, when the pods were beginning to form on the lower branches, and cured in a mechanical drier.

F.C.L.S.—Field cured late soybean hay prepared from plants after the beans were well formed in the pods, but with most of the leaves retained and still green.

A.D.L.S.—Artificially dried late soybean hay prepared from plants after the beans were well formed in the pods, with most of the leaves retained and cured in a mechanical drier.

Representative samples of these eight types of hays were collected, finely ground in a Wiley Mill and then subjected to biological assays. The technique employed for the vitamin A assays was the same as has been previously described (6). The results of these tests are given in Table 1, the values being expressed in Sherman and Munsell (7) vitamin A units.

The Effect of the Hays on Butterfat

Four Guernsey cows divided into two groups of two cows each were used in these feeding experiments. All cows were in the early stages of lactation at the beginning of the feeding trials, and continued through the tests in fairly uniform production. The two groups were fed the respective samples of hay in successive 30 day feeding periods. Group 1 received artificially dried young alfalfa hay (A.D.Y.A.), field cured late soybean hay (F.C.L.S.), artificially dried late soybean hay (A.D.L.S.) and field cured young alfalfa

hay (F.C.Y.A.) in the order named. Group 2 received in order, artificially dried young soybean hay (A.D.Y.S.), field cured late alfalfa hay (F.C.L.A.), artificially dried late alfalfa hay (A.D.L.A.), and field cured young soybean hay (F.C.Y.S.) during the four successive 30 day feeding periods.

The hay was fed daily at the rate of 1.5 per cent of the body weight of the cows. However, some of the cows failed to consume all of the hay offered and as shown in Table 1, there was some variation in the average daily consumption of the different hays. In addition to the hays, the cows were fed limited amounts of corn silage and a balanced grain ration of ground white corn, ground oats and linseed oil meal.

At the end of each feeding period, composite samples of milk (equal amounts from each cow) from each group were collected, and the cream separated and churned into butter. The butter samples were kept in cold storage and portions removed as needed for biological assays.

The vitamin A activity of these samples of butter were determined by biological assays, using the curative method. The results of these assays are shown in Table 1.

TABLE 1

Showing the vitamin A values of alfalfa and soybean hays and the vitamin A activity of butters produced by cows fed the respective hays

	HAY			BUTTER			
	Cow consumption lbs. daily	Vitamin A units		LBS. BUTTERFAT PER DAY	Vitamins A units		
		Per gram	Daily intake (000)		Butter per gram (assayed)	Butterfat per gram (calculated)	Daily output (000)
F.C.Y.A.	12.3	18	100	1.18	29	35	19
A.D.Y.A.	9.7	90	396	1.15	45	55	29
F.C.L.A.	11.5	20	104	1.11	28	34	17
A.D.L.A.	12.8	70	406	1.02	36	43	20
F.C.Y.S.	15.0	36	245	1.06	34	40	19
A.D.Y.S.	10.4	54	254	1.15	38	46	24
F.C.L.S.	12.5	8	47	1.23	19	23	13
A.D.L.S.	16.0	30	219	1.24	24	29	16

DISCUSSION

In the studies on the vitamin A value of the eight samples of hay prepared for these experiments, it may be seen from Table 1 that the vitamin A value of each artificially dried hay was higher than that of the corresponding field cured sample. It may also be noted that in each case the hays prepared from the younger plants were superior to those made from the more mature plants, except in the case of the field cured young alfalfa which apparently had suffered greater vitamin A destruction during the field curing process than the more mature hay. The lower vitamin A value of the field cured young alfalfa as compared with the field cured late alfalfa hay may

be ascribed to differences in the atmospheric conditions during the curing period. The high humidity encountered during the curing of the young alfalfa undoubtedly retarded the rate of dehydration of the hay and favored the greater enzymatic destruction of the vitamin A value of this hay as compared to that of the late alfalfa (3, 6, 8).

Although the marked differences between the artificially dried and field cured samples of alfalfa are as might be expected, based on previous work reported on this subject (2–5), the proportionate destruction of vitamin A in the young soybeans during the field curing process was considerably less than that of the young alfalfa hay. This presents the possibility that the soybeans contain less enzymes than alfalfa which are destructive to the vitamin A activity of hay during the curing process. In the field cured late soybean hay, the proportionate destruction was not as great as has been found for alfalfa, even though the enzyme activity was favored by rather slow drying due to the high content of beans and fibrous stems as well as unfavorable atmospheric conditions.

Evidence previously reported (8) showed that by immediately destroying the enzyme activity of the plants by heat such as used in mechanical driers, alfalfa hay may be produced with the retention of most of the original vitamin A value of the plants. On this basis it seems logical to assume that by similar treatment soybean hay should retain most of its original vitamin A value. If this is true, it becomes evident that soybean plants do not contain as great a vitamin A potency as do alfalfa plants. This is seen by comparing the artificially dried early cut soybean hay with the artificially dried young alfalfa hay, as well as by comparing the soybean and alfalfa hays of later cuttings.

When these hays were fed as the chief source of vitamin A in the ration of dairy cows, the butter which was produced by the cows fed the respective hays, possessed a vitamin A potency similar in relationship to that possessed by the hays. Not only were the artificially dried hays superior to the corresponding field cured samples in producing butters of higher vitamin A value, but with one exception, all artificially dried hays were more effective than the best field cured hay in producing butter of high vitamin A activity. This one exception was in the case of the artificially dried late soybean hay. The butter produced by this hay possessed a lower vitamin A potency than did the butter produced by most of the other hays. This low vitamin A potency was not due to a lack of vitamin A intake by the cows. In Table 1, it can be seen that during this period these cows had an average daily vitamin A intake of 219,000 units and produced butter with 24 units, while cows fed field cured young alfalfa hay had an average daily intake of only 100,000 units, but produced butter containing 29 units per gram. Furthermore, it may be seen that with artificially dried late soybean hay the vitamin A intake was nearly as high as that of the cows receiving the two differently cured

samples of early cut soybean hay, yet the butter produced by the cows fed the artificially dried late soybean hay was significantly lower in vitamin A value than the other two butters. This suppressing action of late cut soybean hay on the transference of vitamin A of the hay to the butter was also observed in earlier experiments (1) and later found to be associated with some factor in the beans (9). However, it should be noted that soybean hay made from the young plants and fed to dairy cows produced butter of high vitamin A activity.

It is interesting to note that Gillian, Heilbron, *et al.* (10) found that although artificially dried grass was much superior to field cured grass in maintaining the high vitamin A activity of summer butter through the winter feeding period, it did not produce butter with exceptionally high vitamin A activity.

In comparing the vitamin A potencies of the various butters reported in Table 1, it is noted that the butter produced by the cows when fed artificially dried young alfalfa contained 45 units per gram which was much higher than any of the other samples; in fact, it possessed the highest vitamin A activity of any butter sample ever tested in this laboratory. Apparently by feeding hay of very high vitamin A content it is possible to increase the vitamin A value of butter even above that produced by cows fed good blue grass pasture. The highest vitamin A sample of butter produced by cows on good blue grass pasture and tested in this laboratory contained 36 units per gram.

However, it is apparent that there is a rather definite limit to the possible increase of the vitamin A in the butter regardless of the number of vitamin A units in the ration. In these experiments, for example, there was four times greater daily vitamin A intake with artificially dried young alfalfa hay than with the corresponding field cured sample, yet there was only a 50 per cent increase in the vitamin A value of the butter.

SUMMARY

1. Comparisons were made of the vitamin A value of artificially dried and field cured alfalfa and soybean hay, cut at two different stages of maturity. Studies were also made of the relationship between the vitamin A activity of the hays and the butters produced by cows fed these respective hays.

2. Artificially dried hays were superior in vitamin A value to the corresponding field cured hays.

3. Hays made from younger plants possessed a higher vitamin A value than did hays made from older plants.

4. Alfalfa plants contain greater vitamin A potency than do soybean plants.

5. Dairy cows when fed artificially dried hays produced butter of higher vitamin A value than when fed field cured hays.

6. Artificially dried young alfalfa hay when fed to dairy cows produced butter of exceptionally high vitamin A value—45 units per gram.

7. Soybean hay made from plants after the beans were well formed in the pods suppressed the formation of vitamin A in the butter sufficiently to produce a butter of only medium high vitamin A activity. Soybean hay made from young plants and fed to dairy cows did not show this suppressing action and produced butter of high vitamin A activity.

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THE SOLUBILITY–FREEZING POINT RELATIONSHIPS OF WATER SOLUTIONS SATURATED WITH RESPECT TO SUCROSE AND DEXTROSE IN RELATION TO THE STORAGE OF SHERBET AND WATER ICE

ALAN LEIGHTON AND ABRAHAM LEVITON
Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

It has long been known that the crusting of sherbet and water ices is due to the crystallization of cane sugar. Leighton (1) showed that, theoretically, such sugar separation could not take place under ordinary conditions if the sherbet was held at a temperature higher than the eutectic temperature of the system cane-sugar-water, which he determined to be as approximately $-12°$ C. Dahlberg (2) showed this independently, and also showed that sugar separation could be prevented at even lower temperatures if dextrose or corn sugar was used in place of part of the cane sugar. Experiment showed him that a concentration of 7% dextrose and 25% sucrose was the most satisfactory for the prevention of sugar separation, although a slightly higher proportion of dextrose could be used. With the realization that the greatest protection was to be had if the proportion of the two sugars to water was that of their ternary eutectic mixture, but lacking suitable laboratory facilities for the precise determination of the joint solubility and freezing point relationships of sucrose and dextrose at temperatures below ordinary room temperature, Dahlberg extrapolated from the data then existing for the relationships at higher temperatures and came to the conclusion that the freezing point of a water solution just saturated to both sucrose and dextrose would be about $-20.0°$ C. and that the proportion of dextrose to sucrose would be approximately that found by him to be most suitable for use in practice, namely, 7 parts dextrose to 25 sucrose, or 1 to 3.57.

While the past few years have witnessed an increased utilization of dextrose in frozen products, the exact solubility and freezing point relationships of water solutions saturated to sucrose and dextrose have not been determined at temperatures below 30° C. This being the case, work was undertaken to determine these relationships, and the data are recorded in this paper.

The procedure best suited for the purpose of determining the eutectic temperature and the composition of the ternary eutectic mixture was to determine the joint solubility in water of corn and cane sugar at a number of temperatures below room temperature, and then to determine the freezing points of a number of these saturated mixtures. The eutectic curve obtained

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by plotting the total sugar concentration against temperature should intersect the freezing point curve obtained from these mixtures at the true ternary eutectic point. In this work the brine temperatures of the refrigeration system were not low enough for the solubilities of cane and corn sugar to be measured at the true eutectic; however, the temperatures obtainable were sufficiently low to permit of extrapolation, and we believe we have located the ternary eutectic temperature with an accuracy of $\pm 0.1°$ C.

For the solubility work a thermostat was used which was equipped with brine coils for cooling and an electric heater for maintaining the desired temperature against the cooling action of the coils. Temperatures could be maintained with an accuracy of $\pm 0.03°$ C.

For the determination of the joint solubilities of the two sugars, equilibrium was approached from under-saturation by rotating within the thermostat 100 cc. glass stoppered bottles containing sugar solution and an excess of the solid phase of each sugar. Rotation was continued until equilibrium was reached, as shown by the analysis of solution taken at intervals of one week. At the lower temperatures a time interval of six weeks was required for the establishment of equilibrium.

When portions of the liquor were to be withdrawn for analysis, the bottles were placed on a rack on the side of the thermostat, and after the solid phase had had time to settle, the liquor was withdrawn by suction into a pipette, the end of which was flared and covered with one layer of filter paper supported by a small perforated platinum disc. In this manner a true sample of about four grams of liquid was obtained by the filtration of the liquor. The actual analyses by means of density and polarimetric measurement upon the diluted sample were carried out in the manner described by Jackson and Gillis (3) and Jackson and Silsbee (4).

The freezing point determinations were conducted in the usual manner using Beckmann thermometers with ice and alcohol as the refrigerant.

TABLE 1

The solubility-freezing point relationships of water solutions saturated with respect to sucrose and dextrose

TEMPERATURE	TOTAL SUGAR	SUCROSE	DEXTROSE	WATER	F.P.
° *C.*	*per cent*	*per cent*	*per cent (anhydrous)*	*per cent*	° *C.*
30.0	73.7[1]	47.1	26.6	26.3	.....
23.3	72.2	48.4	23.8	27.8	.....
9.2	69.5	50.7	18.8	30.5	.....
− 1.3	67.5	51.2	16.3	32.5	−21.20
− 6.5	66.7	51.4	15.3	33.3	−20.10
−12.9	65.7	51.9	13.8	34.3	−18.98
−17.9	64.9[2]	52.2	12.7	35.1	−17.90

[1] Jackson and Silsbee (4).

[2] By extrapolation.

Great care was taken to standardize the procedure and to prevent an undesirable degree of supercooling. The final figures are each the mean of several determinations.

The data are given in Table 1 and are plotted in Figure 1.

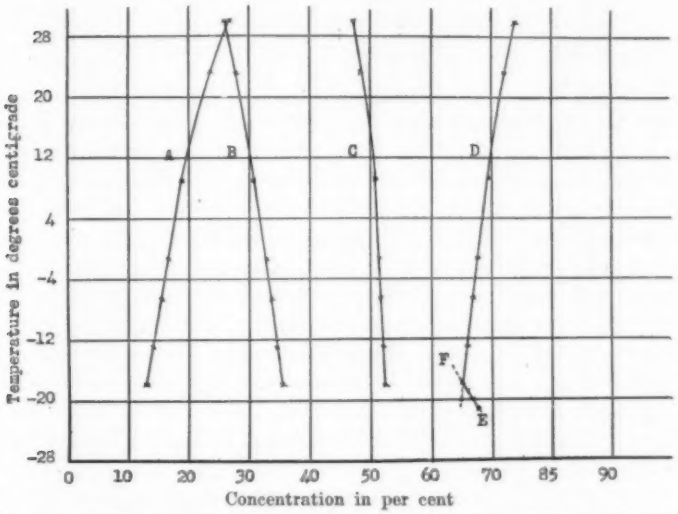

FIG. 1. Solubility-freezing point relationships of water solutions saturated with respect to sucrose and dextrose.

A Dextrose
B Water
C Sucrose
D Dextrose plus sucrose
E Freezing curve
F Eutectic

The ternary eutectic temperature of the system sucrose-dextrose-water is –17.9° C. and the proportion of dextrose to sucrose is 1 part to 4.11 parts.

It is to be noted that this is a markedly lower proportion of dextrose to sucrose than that indicated as permissible in practice. The conclusion is to be drawn, therefore, that in practice a certain degree of supersaturation is permissible, this amount being indicated by a comparison of these data and the figures presented in the work of Dahlberg (2).

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THE AGE THICKENING OF SWEETENED CONDENSED MILK

II. EFFECT OF FOREWARMING CONDITIONS*

V. C. STEBNITZ AND H. H. SOMMER

Department of Dairy Industry, University of Wisconsin

It is a matter of common experience in the evaporated and sweetened condensed milk industries that forewarming conditions, especially temperature, greatly influences the subsequent stability of the finished product. The usual temperature range of 130 to 145° F. at which the milk is condensed in the pan is not sufficiently high to insure destruction of all disease germs, quality-damaging organisms and enzymes that might be present.

A relatively high forewarming temperature is desirable from a biological standpoint especially where the milk is heated in the presence of the sucrose or remnants of previous batches of sweetened condensed milk. Rice (1) has shown that enzymes are more stable toward heat when in the presence of sucrose and that a rancid flavor is apt to develop unless a rather high forewarming temperature is used. He found that in the absence of sucrose, forewarming to 140° F. for 15 minutes or to 150° F. for an instant was sufficient to destroy lipase activity. The forewarming temperature used is further limited by its effect upon the subsequent thickening of the product. The viscosity must be high enough to prevent fat separation but not so great as to be objectionable from the standpoint of the consumer.

In condensery practice a wide range of forewarming temperatures are used and the milk and sugar may be heated together or the sugar solution may be heated separately. A common practice is to heat the milk and sugar with live steam until it begins to boil and then to start drawing it into the pan. These practices have been subjected to experimental study in this laboratory to determine their effect upon the subsequent age thickening of the finished product.

EFFECT OF FOREWARMING TEMPERATURE

The experimental procedure used in these trials has been given in a previous paper (2).

Table 1 shows the progressive age thickening of 8 batches of sweetened condensed milk made from the same milk but with different forewarming temperatures. The milk and sugar were heated together for 10 minutes at the temperature indicated and then promptly cooled to 131° F., the temperature of condensing, before drawing the milk into the pan. For the tempera-

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ture below 203° F. the milk was forewarmed in a water bath, at 203° F. the milk was heated directly with steam, and for the temperatures above boiling an autoclave was used.

TABLE 1

Effect of forewarming temperature—January 23, 1934

°F. TEMP.	135	150	165	180	195	203	230	248
Days				*Viscosity in Poises*				
0	7.4	7.4	8.7	17.3	22.9	19.8	7.4	7.4
2	8.7	7.4	8.7	92.0	*	*	11.55	13.93
6	19.42	16.1	11.55	1584.0			11.55	19.42
11	58.5	19.42	25.8	*			16.75	19.42
16	403.5	13.93	22.3				19.42	22.3
23	403.5	19.42	25.8				19.42	22.3

* Samples too viscous to measure.

Sucrose 44.1%, Fat 7.95%, Milk solids-not-fat 21.43%.

The results show that forewarming temperatures of 150 and 165° F. make a less viscous product than heating the milk to only 135° F. With temperatures of 180° F. and up to boiling the milk becomes considerably less stable, while temperatures above boiling again make the milk less susceptible to age thickening, but produced a dark-brown discoloration in the finished product, especially after a period of storage at 37° C. With the low forewarming temperatures there is danger of fat separation during storage. The excessively high viscosities of the 195 and 203° F. forewarmed milk are greater than is usually the case but are due to the high milk solids content of the finished product as shown at the bottom of the table.

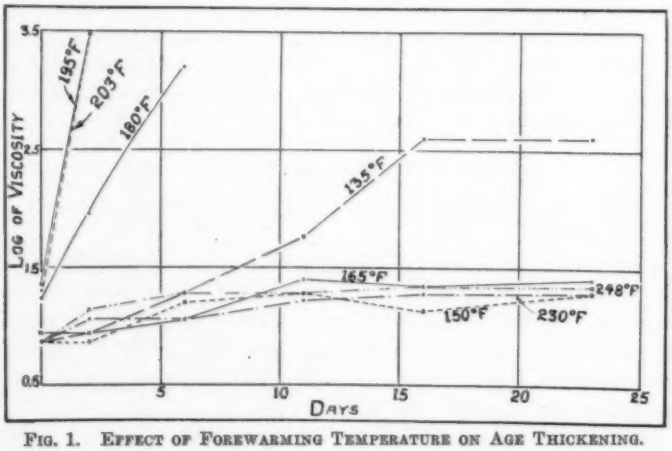

FIG. 1. EFFECT OF FOREWARMING TEMPERATURE ON AGE THICKENING.

Table 1 is plotted in graph 1 and shows that batches made with forewarming temperatures of 150, 165, 230 and 248° F. exhibited about the same tendency toward age thickening.

This trial was repeated with essentially the same results as is shown in Table 2.

TABLE 2

Effect of forewarming temperature—February 15, 1934

°F. TEMP.	135	150	165	180	195	203	242
Days			*Viscosity in Poises*				
0	6.2	6.2	7.4	8.7	8.7	8.7	7.4
4	6.2	6.2	7.4	33.5	393.3	362.9	39.6
8	13.6	9.9	13.6	192.9	*	*	158.9
14	25.8	13.9	19.4	1072.0			505.1
19	25.8	16.8	25.8	1406.0			717.0
25	53.0	22.3	47.5	*			1850.0

* Samples too viscous to measure.

Sucrose 44.1%, Fat 8.46%, Milk solids-not-fat 19.48%.

Visible fat separation in the 135 and 150° F. forewarmed batches.

EFFECT OF PROLONGED HOLDING PERIOD AT FOREWARMING TEMPERATURE

In these trials the milk and sugar were forewarmed together, held for the period designated, and then quickly cooled to 135° F. before drawing the milk into the pan.

The results given in Table 3 are for stable milk and show that as the length of the holding period increases the tendency to thicken becomes greater. In Table 4 a higher forewarming temperature was used and the milk was originally unstable.

TABLE 3

Effect of holding period—February 8, 1934

	NO HOLDING PERIOD	15 MINUTES	30 MINUTES	60 MINUTES
Days		*Viscosity in Poises*		
0	7.4	7.4	7.4	7.4
4	19.42	95.0	125.0	252.8
11	22.3	124.1	221.5	282.6
15	25.8	138.8	163.2	218.8
21	25.8	148.5	173.5	293.0

Sucrose 44.10%, Fat 8.05%, Milk solids-not-fat 20.22%.

Forewarming temperature, 190° F.

From these results it appears that the effect of a prolonged holding period depends upon whether or not the milk is originally stable, the effect being to stabilize unstable milk and to unstabilize stable milk toward age

TABLE 4

Effect of holding period—May 31, 1934

	NO HOLDING PERIOD	15 MINUTES	30 MINUTES	60 MINUTES
Days		*Viscosity in Poises*		
0	31.0	32.0	7.4	7.4
1	1981.0	*	307.5	403.5
4	*		*	*

* Samples too viscous to measure.
Sucrose 44.10%, Fat 8.32%, Milk solids-not-fat 20.15%.
Forewarming temperature 203° F.

thickening. However, the effect is too slight to be of any practical significance in attempting to stabilize unstable milk in this manner. Graph 2 shows the effect of the holding period for both stable and unstable milk.

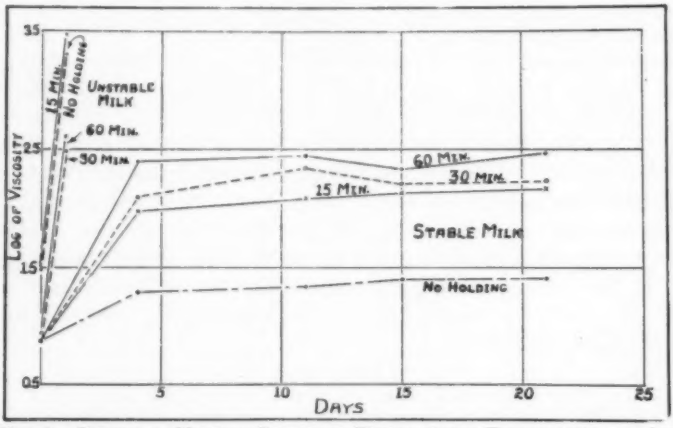

FIG. 2. EFFECT OF HOLDING PERIOD AT FOREWARMING TEMPERATURE ON AGE THICKENING.

EFFECT OF HAVING THE SUGAR AND MILK TOGETHER DURING EXPOSURE TO THE TEMPERATURE OF FOREWARMING AND CONDENSING

The effect of having the sugar in contact with the milk during the forewarming and during the condensing is shown in Table 5. In this trial, the effect of forewarming the sugar syrup and milk separately at 185° F. for 10 minutes, cooling to 135° F., and then mixing the two together before drawing into the pan was compared with adding the sugar syrup near the end of the condensing period. These results are plotted in graph 3.

The results show that having the sugar in contact with the milk during the forewarming period has the greatest effect in causing the milk to thicken

TABLE 5

Effect of sugar on age thickening—March 1, 1934

	A	B	C
Days		*Viscosity in Poises*	
0	7.4	2.76	2.76
5	78.2	33.5	28.4
11	120.0	41.8	28.4
15	114.6	58.5	28.4
22	192.9	83.9	28.4

Sucrose 44.1%, Fat 8.36%, Milk solids-not-fat 20.55%.

A—Sugar and milk forewarmed together.

B—Sugar and milk forewarmed separately, cooled to 135° F., and then mixed together before drawing into the pan.

C—Sugar added as a syrup near the end of the condensing period.

during aging, while having the sucrose in contact with the milk during condensing (131° F. for about 2½ hours) greatly decreases the viscosity, but makes a more viscous product than when the sugar is added near the end of the run. Adding the sugar near the end of the condensing period is likely to produce a product which is too thin and which will show fat separation during storage.

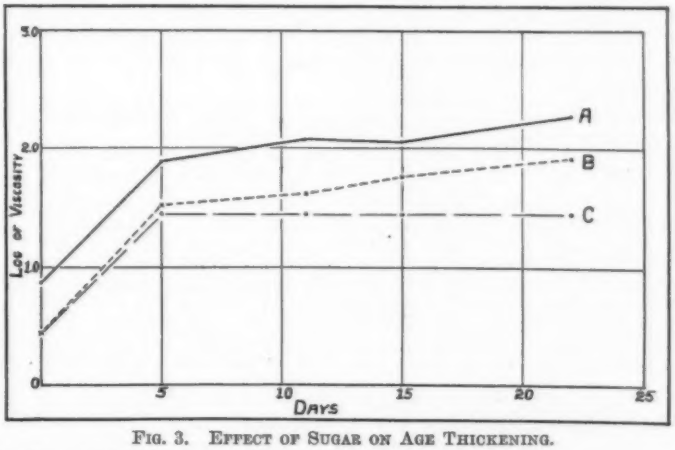

FIG. 3. EFFECT OF SUGAR ON AGE THICKENING.

A—Sugar and milk forewarmed together.

B—Sugar and milk forewarmed separately, cooled to 135° F., and then mixed together before drawing into the pan.

C—Sugar added as a syrup near the end of the condensing period.

DISCUSSION

The method of controlling the age thickening by the use of the proper forewarming temperature is quite satisfactory during the season of the year

when the milk is not originally unstable. A temperature of 175 to 185° F. is quite satisfactory from the standpoint of age thickening and is sufficient to destroy lipase activity, especially if the sugar and remnants of previous batches are not added until after the milk is up to temperature. Forewarming temperatures below 165° F. should be avoided as there is too much danger of fat separation.

A 10 minute holding period at forewarming temperature seems to be quite satisfactory. Longer holding periods as a means of stabilizing unstable milk cannot be recommended.

Where sucrose alone is used as the sweetening agent it should not be withheld from the milk until near the end of the condensing period as is done with corn sugar. In every trial in this laboratory where the sucrose was withheld until late in the condensing period the milk was excessively thin and showed fat separation. In cases where the finished product thickens too rapidly it may be satisfactorily controlled by forewarming the milk and sugar separately, cooling to the pan temperature, and then mixing the two before drawing into the pan.

CONCLUSIONS

Forewarming temperatures of 150 and 165° F. make a product which thickens less rapidly than heating the milk to only 135° F. With temperatures from 180° F. up to boiling the milk becomes considerably more unstable, while temperatures above boiling again make the milk less susceptible to age thickening.

Forewarming temperatures above boiling cause considerable darkening in the finished product during storage.

Prolonged holding periods at the forewarming temperature tend to unstabilize stable milk, but have a slight effect in stabilizing unstable milk.

Having the sucrose in contact with the milk during forewarming has the greatest effect in causing the milk to thicken during storage, while having the sucrose in contact with the milk only during condensing at a temperature of 131° F. greatly decreases the viscosity, but makes a more viscous product than adding the sucrose as a syrup near the end of the condensing period.

Excessive age thickening may be prevented by withholding the sucrose during the time that the milk is at forewarming temperature.

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CAUSES OF DIFFERENCES IN BUTTERFAT PRODUCTION OF COWS IN IOWA COW TESTING ASSOCIATIONS*

MOGENS PLUM
Iowa State College

It is important to know the extent to which differences between production records are caused by environmental factors and how much they are caused by heredity since it is only the hereditary differences which make possible any permanent breed improvement. The present study was undertaken to measure the relative importance of the chief causes of variation among records of cows in Iowa Cow Testing Associations.

SOURCE OF DATA

Through the cooperation of herd owners and of members of the Dairy Extension Service staff at Iowa State College, the record books belonging to men who had been members of a Cow Testing Association for at least three consecutive years, some time during the period 1922 to 1932, were sent to the college where the data desired were copied for this study. Besides the amount of milk and the fat percentage at each monthly test the record books contain, with varying degrees of accuracy and completeness, information about the registration number (or barn number in case of grades) and birth date of the cow, registration number of the sire and dam of the cow, date and age of cow when lactation started, dates when cow went dry and date on which the first test in each lactation was conducted.

The merit of the ration fed to each cow was scored according to an arbitrary scale running from 1 to 9. Grade 1 feeding consisted of legume hay, silage, three kinds of grain including bran, protein supplement, grain while on pasture and grain to dry cows, while grade 9 consisted of non-legume hay and one grain fed whole. All scoring was done by Mr. G. G. Gibson of the Dairy Husbandry Extension staff. The various standards for grading the feeding policy in each lactation are shown on p. 812.

The records from 95 different herds of Guernsey, Holstein and Jersey cattle were used for this study. These herds had been tested regularly in a Cow Testing Association for a period which varied from 3 to 11 years, but averaged 6.8 years. The number and kind of cows under investigation are summarized in Table 1.

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* The writer is indebted to Dr. Jay L. Lush for stimulating suggestions during the conduct of this study and for valuable criticism of the manuscript.

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Standards for Grading

GRADE	ROUGHAGE	GRAIN	NOTES
1	Legume hay and silage	3 grains including bran	a
2	" " " "	2 grains	a
	or Legume hay	3 grains including bran	a
3	Mixed hay and silage	" " " "	a
	or Legume hay	" " " "	a
4	Timothy or mixed hay and silage	" " " "	b
	or Timothy or mixed hay and silage	" " " "	c
5	Mixed hay and silage	2 grains	
6	Timothy hay	3 grains including bran	
	or Silage	2 grains	
7	Non-legume hay and silage	2 grains (whole)	
	or Silage	2 grains (ground)	
8	Non-legume hay	2 grains (whole)	
	or Silage	2 grains (ground)	
9	Non-legume hay	1 grain (whole)	

Grades 1 to 6 required also the inclusion of a protein supplement.

[a] Grain fed when on pasture and grain fed to dry cows.

[b] Grain fed when on pasture.

[c] Grain fed to dry cows.

TABLE 1

Number of cows and number of lactations of cows of the different breeds

BREED	PUREBRED				GRADE				TOTAL			
	Cows		Lactations		Cows		Lactations		Cows		Lactations	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Guernsey	69	3.0	162	2.8	313	13.5	870	14.9	382	16.5	1032	17.6
Jersey ..	850	36.7	1859	31.7	721	31.1	2024	34.5	1571	67.8	3883	66.3
Holstein	220	9.5	553	9.4	143	6.2	392	6.7	363	15.7	945	16.1
Total	1139	49.2	2574	43.9	1177	50.8	3286	56.1	2316	100.0	5860	100.0

ADJUSTMENT OF DATA

The measure of production used in the present study was the total butterfat production in the first eight monthly tests of all lactations which lasted at least 9 months.* If the figures thus obtained are multiplied by the aver-

* This measure was chosen on theoretical grounds as long enough to show some differences in persistency but not long enough to be as seriously influenced by differences in management of C.T.A. herds as 10 month or 12 month lactations would be. The provision

age number of days per month, viz. 30.5, the production for the first eight months of the lactation is obtained.

All records were corrected to a mature equivalent basis using factors obtained from these data. All lactations known to follow abortions were discarded. The record books gave no information concerning the number of times daily the cows were milked, and no corrections could be made for this. In the findings this and other unknown or unrecorded differences in management will appear as causes of variation which in some cases affect all herd mates alike but in other cases will affect only the individual cow.

METHODS OF INVESTIGATION

The data were analyzed by the method of analysis of variance described by Fisher (3). By this method, of which an example in shown in Table 3, it is possible to isolate the variance brought into the data from various sources. When the total variance is to be analyzed into its component parts the observations are classified on the basis of certain criteria which it is supposed may be significant causes of variation. After the variance between the classes has been isolated, a residual variance, commonly called "experimental error," will be left. The relation between the total variance and the residual variance can be expressed either as a correlation between the observations within a class, or as that fraction of the variance caused by the factor, or whole complex of factors, which affects alike all member of the same class.

All the data were punched on cards and computed with the Hollerith tabulating machine.

RESULTS

General time trend

During the 11 years included in the present study the average production increased about 50 pounds of butterfat (on a 10-month lactation basis). However, only 2.8 per cent of the total variance is due to changes in yearly averages. Therefore the time trend which the data show, although significant, is one of the minor causes of variation among these records.

Difference between purebred and grade cows

The average production of all the grade cows (as a total for the first eight monthly testing days) was $10.70 \pm .04$[2] pounds of fat while the average production of the purebred cows was $11.63 \pm .06$ pounds of fat

about the ninth month was used to eliminate cases where the cow was almost dry on the last testing day. However later studies have indicated that it would have made little difference if lactation lengths of 9, 10, 11 or 12 months had been used or even if association yearly records had been used.

[2] In this paper the figures after the $\pm$ are standard errors.

or 8.7 per cent higher. This difference corresponds roughly to a difference of 34 pounds of butterfat for 10 months' production.

This agrees closely with the findings of McDowell (9) who studied over 100,000 yearly records of purebred and grade cows tested in Dairy Herd Improvement Associations. He found that purebred cows excelled grades by 6.7 per cent in production of butterfat but consumed 23 per cent more units of feed than grades. McDowell points out that the higher production of the purebreds might have been due to better feeding.

TABLE 2

Average production of purebred and grade cows by breeds

(Expressed as total pounds of fat produced in the first eight monthly test days)

BREED	AVERAGE PRODUCTION Purebred	Grade	DIFFERENCE PUREBRED ABOVE GRADE	STANDARD ERROR OF MEAN DIFFERENCE*
Guernsey	10.60	10.42	.18	.23
Holstein	12.08	10.73	1.35	.08
Jersey	10.41	11.13	−.72	.17

* The standard error of the difference is figured from the standard deviation within breed (see Table 3), $2.64 \times \sqrt{1/n_1 + 1/n_2}$.

Variance due to breed differences

Table 2 shows the averages by breed. The total variance for the grade records is 5.61 while the total variance for the purebreds is considerably higher, *viz.* 8.50. Both for purebred and grade cows there is a significant difference between breeds. But while 6.18 per cent of the total variance disappears when breed is held constant in the purebreds, only a little more than one half of one per cent of the variance disappears when the same is done for the grade cows. When purebred and grade cows are analyzed together, breed differences account for 2.09 per cent of the total variance, but practically all of this breed variance is caused by purebred cows.

Apparently purebred cows show distinctly more difference between breeds than grade cattle do, and the division of grade cows into different "breeds" may seem unjustified insofar as butterfat production is concerned. Without attempting to give a complete explanation of this situation, two suggestions may be offered. First, part of this lesser variation among the grade cows may be caused by a more vigorous culling of low producing grades than of low producing purebreds. McDowell's (9) study of the records of purebred and grade cows seems to show that there is a more intense culling for grade cows. However, grade cows included in the present study were in these herds long enough to have an average of 2.79 lactations each while the purebreds have only an average of 2.26 lactations each. Second, some purebreds (but not all) are forced by high feeding, extra milking, etc., into unusually high production. Naturally there would be

less of this among grades and perhaps it is a less frequent practice with purebreds of some breeds than others.

Variance due to herd differences

The average production of the different herds varies considerably. The variance within herd is 34.1 per cent less than the intra-breed variance (Table 3). This corresponds to a correlation of .34 between records chosen at random from the same herd in a population of many herds all belonging to the same breed.

TABLE 3

Analysis of variance due to breed, herd, and cow

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	STANDARD DEVIATION
Between breeds	2	877	438.4	
Within breeds	5857	40682	6.946	2.64
Between herds within breed	118	14404	122.1	
Within herds within breed	5739	26278	4.579	2.14
Between cows within herd	2195	16553	7.540	
Between records of the same cow	3544	9725	2.744	1.66
Total	5859	41559	7.093	2.66

All differences are highly significant ($P < .01$).

Portion of total variance due to differences between:

Breeds $\frac{7.093 - 6.946}{7.093} = 2.09\%$.

Herds $\frac{7.093 - 4.579}{7.093} = 35.4\%$.

Cows $\frac{7.093 - 2.744}{7.093} = 61.3\%$.

Portion of intra-breed variance due to differences between:

Herds $\frac{6.946 - 4.579}{6.946} = 34.1\%$.

Cows $\frac{6.946 - 2.744}{6.946} = 60.5\%$.

Portion of intra-herd variance due to differences between cows:

$\frac{4.579 - 2.744}{4.579} = 40.1\%$.

Records from the same herd may be either from the same cow, but made in different years, or they may be from different cows and made during the same or in different years. Herd mates may be related or may be somewhat alike because each owner has selected or culled them according to his own ideas. In order to exclude the cases where cows have more than one record, the analysis of variance of the first available records of the 2316 cows was carried out in a manner similar to the one shown in Table 3. The variance due to herd differences was 33.0 per cent of the intra-breed variance. This

corresponds to correlation of .33 between first available records of herd mates. The smallness of the difference between 33.0 and 34.1 is not surprising since two records chosen at random from a large herd would rarely be records of the same cow.

The important herd effect shown by this correlation of about one third may have several causes. Each herd may be a distinct group with a certain uniformity, obtained either by selection or by consanguinity among the animals. Such selection and culling may not have been toward the same ideal nor with equal intensity in all herds. The environment and management may be rather uniform for all cows in the same herd but distinctly different from herd to herd. Gowen (7, pp. 278–298) using Holstein-Friesian Advanced Registry data found that the correlation ratio between herd and milk yield was .603 and the correlation ratio between herd and butterfat percentage was .490. If the degrees of freedom are taken into consideration these correspond to correlations of .33 for milk and .20 for butterfat percentage between records of herd mates. Although Gowen's figures come from Advanced Registry data, they show a herd effect of surprisingly near the same size as was found in these Iowa Cow Testing Association herds.

Variance due to differences between cows

Table 3 shows that 40.1 per cent of the intra-herd variance and 60.5 per cent of the intra-breed variance are due to differences between cows. (Since in these data all records of a cow were made in the same herd, the intra-breed differences between cows include the herd difference.) This corresponds to a correlation of .40 between records of the same cow in a population of cows all belonging to the same herd, or to a correlation of .60 between records of the same cow in a population of cows kept in many herds. In a single Jersey herd Gowen (5, 6) found an average correlation of .54 and .52 respectively, between milk production and fat percentage of one lactation at any age, and milk production and fat percentage of another lactation at another age. In a population of cows from many herds Gowen (7) found an average correlation of .67 between successive 365-day milk yields of Holstein-Friesian cows and an average correlation of .70 between similar yields of Guernsey cows. Likewise in a population of Danish cows from many herds, Gaines and Palfrey (4) found for yield of fat-corrected-milk (milk energy) an average correlation between successive lactations of .50. Each of those cows had been kept long enough to have ten normal lactations. Probably few really low producers among those which started on test would have escaped culling this long. The correlation of .60 found in the present study is somewhat higher than the average correlation found by Gaines and Palfrey, but somewhat lower than the correlation found by Gowen.

Breed, herd, and cow differences together account for 61.3 per cent of

the total variance. The remaining 38.7 per cent of the variance is due to differences between the records of the same cow. These differences may be caused by a number of factors, such as year to year changes in weather, management and feeding, length of preceding dry period, season of calving, imperfections in the corrections for age, errors in assuming that the actual production during the eight months was accurately shown by the monthly tests, and perhaps other factors. Even after allowance has been made for feeding, length of dry period, season of calving and other recorded factors of management, there will be a considerable residual variance left, the cause of which cannot be determined precisely. For convenience such causes may be lumped together as unrecorded or unknown variations in the environment.

Influence of feeding

The feed the cows received was scored according to an arbitrary scale planned to run from 1 to 9, but only two cows' feeding actually was scored lower than grade 7. Cows receiving grade 1 feeding produced approximately 490 pounds of butterfat for a 10-month period while cows receiving grade 7 feeding produced only about 276 pounds of butterfat. The analysis of variance shows that there are significant differences between the production of cows grouped in the different feed grades. When the differences in average production which are associated with differences in feed grade are eliminated the variance is reduced 16.8 per cent. However the correlation between feed grades of the same cow in consecutive years is .78. This correlation is made up of a correlation of .92 between means of herds and a correlation of .59 within herds. Therefore there are marked differences in the feeding policy from herd to herd and there is some tendency to feed the same cow the same way year after year. This indicates that a considerable part of the differences which exist between herd averages is associated with recorded variations in their feeding practices. Doubtless there were other important but unrecorded differences in feeding practices. Of the intra-herd variance 8.3 per cent (which equals 5.4 per cent of the total variance) was due to variations in feeding grade. The variance associated with feeding grade (16.8 per cent) may then be divided into one part (5.4 per cent) due to unlikenesses in feeding within herds and another part (11.4 per cent) due to differences in the feeding policy from herd to herd.

The condition of the cow at calving is usually considered to be an important factor in determining her production, at least during the first part of the lactation. An analysis of variance shows that the feed grades of the previous year and that of the year the record is made, together are responsible for 1 per cent more of the total variance than is the feed grade the year the record is made. Apparently there is some after-effect of the previous year's feeding, so far as that is different from the current year's

feeding, but this effect is small compared with the total variation when allowance has already been made for the feeding the year the record is made.

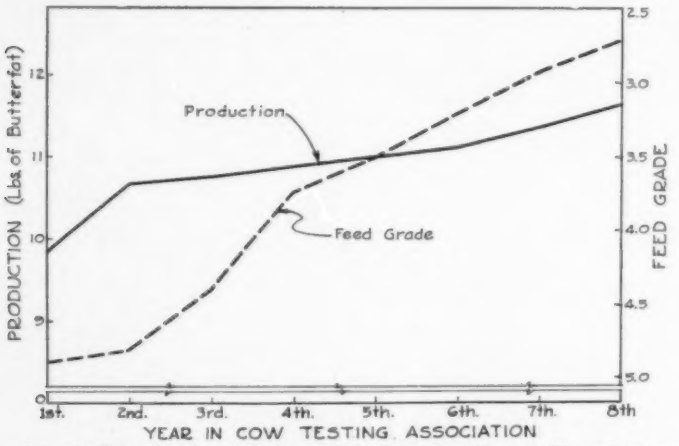

FIG. 1. AVERAGE PRODUCTION AND AVERAGE FEED GRADE FOR COWS IN HERDS WHICH HAVE BEEN IN COW TESTING ASSOCIATIONS FOR AT LEAST 8 YEARS.

The two scales are so chosen that one standard deviation on each occupies the same distance on the chart. Thus the slopes of the two lines show comparably their regression on the herd owner's experience in the cow testing association. Experience has a marked influence upon feed grade while the progress in production is slower, except during the first year.

Influence of herd owner's experience

Figure 1 shows that the average production of herds which have been in Cow Testing Associations for at least eight years gradually increases. The largest increase takes place from the first to the second year. Figure 1 also shows that the feeding improves rather steadily from year to year. Ten and three tenths per cent of the intra-herd variance disappears when the effect of yearly variations are eliminated. This 10.3 per cent is not very different in size from the 8.3 per cent of the intra-herd variance which is caused by differences in feeding. This may be largely accidental, but the similarity of the trends in Figure 1 indicates that the improvement in feeding as the owner's experience increases is an important factor in causing year to year variations within a herd.

Influence of the length of the preceding dry period

Investigations concerning the influence of the length of the dry period preceding lactation have usually shown that production is lowered when the dry period is extremely short. Most investigations have also shown

that very long dry periods tend to decrease the production in the following lactation. Thus there seems to be an optimum length of the dry period, usually given to be one to three months in length. Quite similar results were obtained in the present study but, excluding heifers, there were only 100 records which had no dry period at all. Slightly more than 1 per cent of the total variance disappeared when the effects of variation in dry period were removed. Apparently length of dry period is a minor cause of variation among these records.

Influence of season of calving

In the present data there are differences in production of cows calving at different seasons of the year. Cows calving during November to January produced 13.6 per cent more butterfat than cows calving in May to July, which is in close agreement with Cannon's (1) studies of more than 68,000 records from Iowa Cow Testing Associations. However, only 3 per cent of the total variance is due to differences in the season of calving. Thus although the season of the year has a significant influence upon production it is a minor cause of variation here.

The relative importance of heredity and environment

Gowen (8) has studied the relative importance of heredity and environment on milk production and butterfat percentage of Jersey Register of Merit cattle. He finds that the inheritance of the cow determines 50–70 per cent of the variation in milk production. Environment common to sisters accounts for 5 to 10 per cent and other factors peculiar to the cow herself account for 20–45 per cent. In the butterfat percentage the inheritance controls 75–85 per cent, the environment common to sisters has no apparent effect and the influences common only to the cow herself control 15–25 per cent. However in these calculations Gowen assumed that common environment contributed nothing to the correlation between daughter and dam. Such an assumption seems too extreme in view of the differences in herd management. Perhaps that goes far toward explaining the highness of the values he finds for the importance of heredity.

Wright (12) has calculated from Gowen's data from Holstein-Friesian and Guernsey cattle that milk production for these cows is determined about 75 per cent by heredity. Likewise Wright has calculated that the average correlation between the genotypes of the cows in a herd is about .20. This also interprets the differences between herd averages as much more genetic than environmental.

For cows tested under the variety of conditions found in Iowa Cow Testing Associations the statistics need not necessarily be the same as for cows on official test. There might be a wider range of environments (or a nar-

rower) in Cow Testing Association data. Likewise there might be a much wider or narrower range in the real hereditary productivity of the cows in the Cow Testing Association population.

In Table 3 it was shown that there is an intra-herd correlation between one record of a cow and another record of the same cow of .40. Now if heredity were the only thing which tended to make the records of the same cow alike, the portion of the intra-herd differences caused by heredity (h^2) would be .40. Undoubtedly there are other factors which cause two records of a cow to be the same, as for instance intra-herd correlations between the environment to which the same cow has been exposed in the different years and intra-herd correlations between the cow's environment and her heredity. Such a correlation will exist if there is a tendency to feed individual cows according to production, but it has not been possible to measure it from the present data.

Hereditary differences between cows thus cause something less than 40 per cent of the variance found within a herd.

The influence of common herd environment

Possibly some herds are fed well because they produce well rather than the reverse, and therefore it may not be correct to say that the differences associated with differences in feeding are *caused* by differences in feeding. But with this qualification the feeding practices of the herds can be considered as a cause of variation between herds. However this accounts for only part of the differences between herds. The herds may differ in unrecorded ways in their feeding or in other important features of their management, such as times milked per day. On the other hand there doubtless are differences in the average intrinsic merit of the cows in different herds. The things which make herd mates resemble each other in productiveness may thus be partly hereditary and partly environmental.

The correlation of .33 between records of herd mates is a measure of the extent to which the combined genetic and non-genetic causes make them produce alike but tells nothing about how much of this is genetic and how much non-genetic. No satisfactory and simple answer to this was found. The writer's opinion is that differences between the average environment of different herds account for approximately three-fifths of the variance between herds. This figure is highly tentative, since it has not yet been possible to determine its value within narrow limits. The remaining two-fifths of the variance between herds can tentatively be regarded as due to hereditary differences in the average kind of cows in the different herds.

Discussion of results

The influence of common herd environment on correlations between relatives is a factor which should not be overlooked when aggregates of data from many herds are analyzed together.

The correlation between daughter and dam was .31 when the first available record of each was used and the data were analyzed together without regard to herd differences. The corresponding correlation within herds was .06 (based upon 683 dam-daughter pairs from 81 herds). Neither intra-herd assortative mating nor common environment can be very important in this intra-herd correlation. Doubling this correlation furnishes a rough estimate of the importance of heredity within the herd. However, only that fraction (g^2) which can be expressed additively of the variance (h^2) due to gene differences and combinations, enters into the daughter-dam correlation. This analysis therefore gives $h^2g^2/2 = .06$ or $h^2g^2 = .12$. If dominance is the major cause of non-additive combination effects of genes, it is suggested by Fisher (2) that g^2 is typically around $\frac{2}{3}$ while Wright (11) for other reasons thinks that g^2 is more typically around $\frac{4}{5}$. These two values would give values for h^2 of .18 and .15 respectively. If non-additive interactions of non-allelomorphic genes (epistatic effects) are frequent and of considerable magnitude, g^2 may be lower. Consequently h^2 would take higher values than these, although it could not go above the .40 indicated by the intra-herd correlation between different records of the same cow.

In 246 cases (scattered through 68 herds) the dams had a record starting not more than 3 months from the time the daughter's record started and each dam also had an earlier record. When the first record was used for both daughter and dam, the total correlation was .32 while the correlation within herds was .10. For contemporary records of daughter and dam the total correlation was .40 and the correlation within herds was .27, showing a considerable effect of simultaneous environment. The total correlation between the two records of the dams was .41 while the correlation within herds was .25 for this. The correlation of .10 between the first available record of both daughter and dam gives, for values of g^2 of $\frac{2}{3}$ and $\frac{4}{5}$, values for h^2 of .30 and .25 respectively. Lower values of g^2, which would occur if the effect of dominance is more than suggested by Wright and Fisher, or if epistatic effects are important, would give higher values of h^2.

Some other data,[3] collected in connection with the proved sire work in Iowa Cow Testing Associations, may be used for an independent estimate of the genetic part of the variance in butterfat production. These data consist of the records of 2,394 daughter-dam pairs used in proving 355 sires. An analysis of these records shows a total correlation of .408 and an intra-sire correlation of .178 between records of daughters and dams. These records were either lactation records or C. T. A. yearly records, corrected for age by the 70, 80, 90 per cent rule. Some of these records may have been made during the same year and there may have been other than genetic

[3] Kindly furnished by E. N. Shultz of the Dairy Husbandry Extension Service.

causes for this correlation. If genetic causes alone are responsible for this intra-sire correlation the genetic part of the variance (h^2g^2) is at the most .36, which is slightly higher than the values found in the present study.

These correlations and the conditions under which they are obtained seem to make it likely that h^2 within herds may be somewhere between .20 and .40. Until further studies have been made the value of h^2 may be set at somewhere around one third to one fourth. This figure is not exact enough to be very satisfactory. Nevertheless it does explain and make reasonable a host of breeders' practical experiences which have hitherto seemed puzzling. It explains especially the many individual disappointments in selecting for high production in contrast with the favorable general experience with selection as a slow but fairly dependable average means of improvement. It is highly desirable that there be more studies to determine the value of h^2 more closely. Especially would it be desirable to get more evidence about g^2 on which the evidence here (only that involving correlations between relatives) is scanty. In measuring the immediate consequences of culling, h^2 is important as expressing the portion which is real of the apparent difference between the productivity of those culled and those kept. But in selecting for breeding purposes, h^2g^2 is more important since it is the fraction of superiority in the parents (as indicated by their records) which may be expected in their offspring.

The hereditary part of the herd differences (about 40 per cent as a very rough approximation) and the hereditary part of the intra-herd variance (one third) are not far from the same magnitude. Since both are approximations it may be well enough for practical purposes to combine them into a single value. A value of $\frac{2}{5}$ would probably give a maximum estimate of the hereditary part of the variance among single records from many herds while a value of $\frac{1}{3}$ would be more conservative.

The influence of year to year changes within a herd (probably mostly environmental but partly genetic, since some cows are culled and others added each year) accounts for 6 or 7 per cent of the total variance or roughly 10 per cent of the intra-herd variance. If this year-to-year variation were discounted by figuring production records within a herd as deviations from the yearly average, the resulting figures would doubtless come closer to representing hereditary differences in productivity, *i.e.*, h^2 would be a little larger in them. However, yearly variations are not nearly so important in these Iowa records as they are said to be in the data used in Germany by von Patow and his associates (10) who seem to consider year-to-year variations as the major environmental cause of variation. Moreover the measurement of productivity as so many per cent above or below the herd average offers many practical difficulties in comparing cows which make records in different herds or in different years in the same herd, especially if the herds are small.

The estimate of the genetic part of the variance obtained from the present study is lower than the values given by Wright and by Gowen. Wright's unpublished computations (on Gowen's data from Holstein-Friesian and Guernsey Advanced Registry) seemed to indicate that nearly all the likeness between herd mates was genetic rather than due to common herd environment. This rested on the smallness of the difference between half sister correlations and non-sister herd-mate correlations as contrasted with the higher correlations between full sisters and between dam and daughter. Gowen's estimate of the magnitude of the environmental influence is what is called intra-herd year-to-year variations or "seasonal environment" in the present paper. If what seems to be the environmental part of the herd differences here (about 20 per cent) is subtracted from Gowen's figure for the influence of heredity, values are obtained which are only slightly higher than the values (one third) found in the present study. Gowen's estimate of the influence of environment (5 to 10 per cent) corresponds closely to the comparable value for intra-herd year-to-year changes in environment (about 10 per cent) found in the present study.

The correlations between records of the same cow reported by Gaines and Palfrey (4) and Gowen (7) would give values for the hereditary part of the variance which are only slightly different from the one found in the present study if environmental causes of differences between herds accounted for about 20 per cent of the total variance. Thus a value of about one third for the hereditary part of the variance (h^2) should not seem surprisingly low.

It seems likely that the genetic part of the variance which enters into parent offspring correlations (h^2g^2) is not far from one fourth. The practical consequence of such a value of h^2g^2 would be that if the cows selected as parents for the next generation average 100 pounds of butterfat above the level of the breed or herd and the bulls are equally superior, then the offspring would average about 25 pounds above this same level. If the average interval between generations is 5 years this would correspond to an improvement of 5 pounds of butterfat per year. This rate of improvement could be increased if selection could be made more intense or if the genetic part of the variance could be increased, *i.e.*, if other sources of variation can be controlled or taken into consideration by making proper corrections so that the breeder is no longer deceived by their effects.

While the results here are only approximate quantitatively, no reason is seen for thinking that they are biased either in the direction of largeness or smallness. Additional studies on suitable data with further refinements to verify and extend the conclusions of this study are to be desired. If and when such verifications are obtained, the conclusions can be the basis of improved practical procedure, especially in planning what is to be gained by this or that method of selection and in deciding how much attention to

pay to pedigree, to the animal's own production and to the production of its various progeny.

TABLE 4

Relative importance of causes of variation in butterfat production

CAUSES OF VARIATION	PER CENT OF TOTAL VARIANCE	
Breed		2
Herd		
Feeding policy of herd	12	
Other causes (genetic or environmental)	21	33
Cow (mostly genetic)		26
Residual (year to year variations)		
Feeding variations within the herd	6	
Other year to year differences	1	
Length of dry period	1	
Season of calving	3	
Other factors	28	39
Total		100

SUMMARY

The importance of different sources of variance is summarized in Table 4.

Practically all of the 2 per cent due to breed was found among the pure-bred cows, the grades showing very little breed difference.

Differences between cows (26 per cent) are mostly hereditary, but include the effects of any permanent change taking place in the cow before she starts giving milk and the effects of continually giving one cow better or poorer feeding and management than her herd mates.

Variation in butterfat production among these cows seems to have been determined about one third by differences in their heredity. The major source of uncertainty in this estimate is the question of what part heredity plays in the 21 per cent of the variance arising from herd differences not connected with recorded feeding practices.

The large amount (28 per cent) of unexplained variance indicates the considerable discrepancies still to be expected between a cow's record and her real producing ability, even after careful corrections have been made for age, feeding, dry period, calving season and the general level of the herd in which she is kept.

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SUBSTANCES ADSORBED ON THE FAT GLOBULES IN CREAM AND THEIR RELATION TO CHURNING. IV. FACTORS INFLUENCING THE COMPOSITION OF THE ADSORPTION "MEMBRANE"*

CHARLES E. RIMPILA AND L. S. PALMER
Division of Agricultural Biochemistry, University of Minnesota, St. Paul

INTRODUCTION

Hattori (1), Palmer and Wiese (2), Wiese and Palmer (3) and Palmer and Lewis (4) have shown that the protein fraction of the natural fat globule "membrane" in milk is different chemically and physically from any of the known proteins of milk. Palmer and Samuelsson (5) and Palmer and Wiese (2) have demonstrated that the natural fat globule "membrane" also contains phospholipides which consist chiefly of a fraction resembling a lecithin.

The work of Wiese and Palmer (6) has shown that both "washed" cream and an artificial emulsion of butter-fat in buttermilk from sweet cream behave like normal cream in respect to churning, but that other artificial creams prepared with calcium caseinate, whey, blood globulin, phospholipide, or blood globulin plus phospholipide do not churn normally. It is thus demonstrated that the natural fat globule "membrane" is not formed when preparing artificial emulsions of butter-fat by means of the major constituents of milk plasma. Nevertheless, North and Sommer (7) erroneously assume that the natural interfacial material is concerned in the determination of the zeta potential between butter-fat and diluted skim milk.

The present paper deals with the isolation and comparison of the analyses of the fat globule "membrane" and its protein fraction from artificial emulsions of butter-fat in skim milk, buttermilk, whey, and casein solutions together with analyses of the natural fat globule "membrane" and its protein fraction.

EXPERIMENTAL METHODS

All the creams, both natural and artificial, were washed by the method described by Wiese and Palmer (6).

The fat globule "membrane" materials were isolated as follows: The cream after washing was churned after cooling and tempering at a natural churning temperature. The free buttermilk combined with the buttermilk

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from the melted butter was supercentrifuged. The supercentrifuged buttermilk was dried at 40° C. under reduced pressure, the dried residue constituting the crude fat globule "membrane."

The protein portion of the "membrane" was the residue remaining after exhaustive extraction with absolute ethyl alcohol and ether.

The artificial emulsions of butter-fat were prepared by passing a mixture of melted, filtered butter-fat and the emulsifying solution at 38° through a Gaulin homogenizer five times at 200 pounds pressure. This treatment produced a "milk" in which the fat globules were similar in size to those found in natural milk.

The protein content of the "membrane" was determined by weighing the residue remaining after alcohol and ether extraction of a weighed sample of crude "membrane."

Aliquots of the alcohol-ether extract were ashed with $MgNO_3$, following the method of Wiese, Nair and Fleming (8) and the phospholipides calculated by multiplying the phosphorus content of the ash by 100 and dividing the result by 3.85 on the assumption that lecithin was the only phospholipide present. The assumption probably permits reasonably accurate comparisons between the different "membranes" studied.

The protein content of the washed cream was determined by weighing the residue left after treating a weighed sample of cream with dioxan[1] (ratio 1:7), filtering, extracting with ether and drying.

All phosphorus analyses were performed by the Fiske and Subbarow (9) method. Lipide phosphorus in cream was determined by the method of Wiese, Nair and Fleming (8).

Phosphatase activity of the creams was determined by the method of Kay and Graham (10).

The method reported by Cavett (11) was used for the Van Slyke nitrogen distribution of the "membrane" proteins.

DESCRIPTION OF THE CREAMS AND THEIR TREATMENT

Natural creams. Cream 1.—Two quarts of fresh cream were washed four times with distilled water. A sample of cream was taken after each

[1] Dioxan is a trade name for 1–4 dioxane (tetra-hydro-p-dioxin, or diethylene dioxide) manufactured by the Carbide, Carbon Chemicals Corporation, New York City. It is a good fat solvent and is miscible with water in all proportions. These facts make it useful in isolating the protein from cream. Preliminary experiments with mixtures of butter-fat, water and dioxan showed that the least amount of dioxan necessary to form a miscible mixture with 1 gram of a 50 per cent cream was 7 cc. and less dioxan was required for the higher fat creams. Since most washed creams contained over 50 per cent fat it was decided to use the ratio of 1 gram cream to 7 cc. dioxan in making the fat globule "membrane" protein analyses, and for creams with less than 50 per cent fat more dioxan was added until a miscible mixture was formed, when the samples were filtered. Although the dioxan dissolved the phospholipides and lipides, it was deemed necessary to extract the protein with ether in order to remove the last traces of these substances.

washing and saved for total phosphorus, lipide phosphorus, and protein analyses. The fat globule "membrane" was not isolated from this cream.

Cream 2.—One gallon of fresh cream was washed with distilled water. One quart was taken out after the fourth, sixth, eighth and tenth washings, and they were used for isolation of the fat globule "membrane." The total phosphorus, lipide phosphorus, and protein were determined in the creams washed four, eight, and ten times. The cream washed six times was lost.

There was not enough "membrane" protein isolated from the samples of creams that were washed four and eight times respectively to determine the Van Slyke nitrogen distribution; however, enough "membrane" protein was obtained for this determination from the sample of cream washed ten times.

Cream 3.—One gallon of fresh cream was washed four times with distilled water. The fat globule "membrane" was then isolated and analyzed.

Cream 4.—One gallon of fresh cream was washed six times with distilled water, and the "membrane" isolated and analyzed. A sample of the original cream and samples from the first, fourth, and sixth washings were saved for phosphatase determinations.

Cream 5.—Fresh cream was washed with distilled water twenty-four times and samples withdrawn at the fourth, eighth, twelfth and twenty-fourth washing. The samples, together with one of the original cream, were preserved with formaldehyde, adding about 1 part HCHO per 2,500 parts cream.

Cream 6.—Fresh cream was washed eight times and samples withdrawn for analysis after the fourth and last washings. The samples, together with a sample of the unwashed cream, were preserved with formaldehyde (1:2,500).

Cream 7.—This was a repetition of cream 6 except the samples were not preserved with formaldehyde.

Cream 8.—Two quarts of fresh cream were washed four times with distilled water, then four times with sweet rennet whey, and finally four times with water. Sweet rennet whey was prepared by adding one and one-half times the required amount of rennet to fresh skim milk, cutting the curd with cheese knives, cooking it slightly, and finally drawing off the whey. This whey was immediately centrifuged to remove the casein particles, cooled, and stored in a refrigerator until used the next day. Before the whey was used for washing the cream, it was warmed to 38°–43°.

Samples of cream were saved from the following washings; fourth whey washing, and the first, second, third and fourth water washings following the whey washing. These samples were analyzed for total phosphorus, lipide phosphorus, and protein. The sample of cream which had been washed twelve times (four times by water, four times by whey, and again four times by water) was used for isolation of the fat globule "membrane."

Artificial creams. Cream 9. Whey cream.—The sweet rennet whey used

in making this cream was prepared by the same method as that described for the whey used to wash cream 8. Butter-fat was obtained by filtering melted butter. The emulsion of butter-fat in whey was made by the method described under experimental methods.

The whey cream was washed twice without difficulty, but during the third washing, the cream clogged the centrifuge bowl and had to be scraped out and redispersed in water. This redispersed cream constituted the third washing and was used for isolation of the "membrane." No attempt was made to wash the cream more than three times.

Cream 10. Skim milk cream.—The skim milk used for the preparation of this cream was obtained from the separation of fresh whole milk. It was used immediately for making the butter-fat emulsion.

The cream thus obtained was washed twice without trouble, but both the third and fourth washings had to be scraped out of the centrifuge bowl and redispersed in water. Samples were taken from the original cream and from the first, second, third and fourth washings, and were used to determine their total phosphorus, lipide phosphorus and protein contents. A sample of the fourth washing was also used for isolation of the "membrane" material.

Cream 11. Buttermilk cream.—The buttermilk used for the preparation of this cream was obtained from the churning of fresh unpasteurized sweet cream. It was then supercentrifuged to remove all traces of unchurned fat globules. Butter-fat was obtained from filtered melted butter.

The buttermilk cream was washed three times but the third washing of the cream had to be scraped from the centrifuge bowl and redispersed in water. No attempt was made to wash the cream more than three times. The redispersion of the cream from the centrifuge bowl constituted the third washing which was analyzed for total phosphorus and lipide phosphorus, and also used for the isolation of the fat globule "membrane" protein. Samples of the original cream and of the first and second washings were analyzed for total and lipide phosphorus.

Cream 12. Buttermilk cream.—Buttermilk used for this experiment was prepared by churning two gallons of unpasteurized cream in a metal barrel churn. The buttermilk was then centrifuged to remove unchurned fat globules and particles of butter-fat. The method for preparing this cream was not the same as that used for making the other artificial creams. Instead of emulsifying butter-fat into buttermilk to produce a four per cent milk, sufficient butter-fat was used to make a cream having thirty-five per cent fat. This cream was washed twice without much difficulty, but the separation of the third washing clogged the centrifuge bowl. The cream from the bowl was redispersed in distilled water and was used for the isolation of the fat globule "membrane."

Cream 13. Casein cream.—The casein was prepared from skim milk by the Van Slyke and Baker (12) method modified by Van Slyke (13). A solu-

tion containing 2.5 per cent casein was made by dissolving the casein in saturated lime water and titrating this solution to a pH 6.6 with one per cent H_3PO_4. This casein solution was mixed with enough butter-fat to form a thirty-five per cent cream, and the mixture emulsified.

The casein cream could not be washed with water as it clogged the centrifuge bowl during the first washing.

EXPERIMENTAL RESULTS

The effect of washing cream with water upon its lipide phosphorus, total phosphorus, and protein content. The results of these experiments are shown in Table 1. After the third washing the decrease in the above mentioned constituents in cream 1 was very small. There was further gradual decrease of less magnitude in lipide phosphorus and total phosphorus after the fourth washing, which continued until the emulsion broke. This fact is illustrated in the experiments with cream 11. However, after the twenty-fourth washing the cream that remained still contained the fat globules with the "membrane" intact, but weakened. The "membrane" material is held very tightly to the fat globules.

The lipide phosphorus analyses 1, 2 and 3 in Table I were made on the same sample of cream at intervals of about a week or more. These tests were made to investigate the possible deterioration of these "membrane" constituents upon standing. There was a decrease in lipide phosphorus in creams 2 and 5, but none in creams 6 and 7. Since formaldehyde was added to cream 6 but not to cream 7, these contradictory results leave the question of "membrane" lipide deterioration open to further investigation; they suggest that the "membrane" may at times carry a lecithinase.

The washed artificial creams contained very little lipide phosphorus, showing that the "membrane" was not typical of that found in natural creams.

Cream 8 washed four times with water, four times with whey, and finally four times with water, had a final lipide phosphorus content lower than that observed for cream 5 washed twelve times with water. One of the major effects of washing with whey was to decrease the lipide phosphorus content of the "membrane."

All the natural creams withstood washing with water even up to twelve times as is shown by cream 5. This cream began to break after the twelfth washing but some cream was left even after twenty-four washings.

The artificial creams could not be washed more than three times at the most, which is further evidence that their "membranes" were not typical of natural cream.

The effect of washing cream on its phosphatase activity.—The effect of washing cream on its phosphatase activity is shown in Table 2. After washing six times with distilled water the cream still contained more than 50 per

cent of its phosphatase activity. During the churning of the washed cream the phosphatase evidently goes into the buttermilk as is shown by the high enzyme content of the buttermilk from melted butter. This shows definitely that part of this enzyme in cream is a constituent of the fat globule "membrane" and can not be washed away with water, and substantiates the conclusions of Kay and Graham (10) based on the phosphatase activity of various fractions of milk, whole milk, skim milk, cream, butter, buttermilk.

Composition of the fat globule "membrane."—Table 3 gives the analyses of the crude fat globule "membranes" isolated from washed natural cream. The range in composition of the fat globule "membranes" is as follows: Protein 21.98 to 43.96 per cent, phospholipides 13.00 to 26.00 per cent, and non-phospholipide ether-extractable fraction 56.04–78.02 per cent. The "membrane" from artificial creams contained only traces of phospholipides, but contained 44.66 to 55.25 per cent protein and 44.75 to 55.34 per cent non-phospholipide ether-extractable material.

Composition of the fat globule "membrane" protein.—Table 4 contains the results of all the "membrane" protein analyses performed compared with similar analyses of the "membrane" protein previously published from this laboratory.

Comparison of the natural fat globule "membrane" proteins with each other.—There are small significant variations in total nitrogen, Van Slyke nitrogen distribution, and phosphorus content among the different natural cream "membrane" proteins, but as a group they compare favorably with those isolated by Hattori (1) and Wiese and Palmer (3). It is evident from these analyses that the "membrane" protein is not one of the common milk proteins. The variations in analyses of the "membrane" protein which were found in this study indicate that the methods employed for its isolation do not result in a "pure" protein but rather a protein contaminated by varying although minor amounts of the other proteins of milk.

Comparison of the natural fat globule "membrane" protein with those isolated from artificial creams.—The Van Slyke nitrogen distribution and phosphorus analyses show definitely that the fat globule "membrane" proteins of the artificial creams, are not the same as those isolated from natural creams.

Comparison of the "membrane" proteins among the individual artificial creams.—The "membrane" proteins from creams 11 and 12 (buttermilk creams) are very similar to each other in all respects, but differ from the "membrane" proteins isolated from cream 9 (whey cream) and cream 10 (skim milk cream). The "membrane" protein from cream 9 (whey cream) resembles in its Van Slyke nitrogen distribution a mixture of lactalbumin and globulin, and that from cream 10 (skim milk) a mixture of casein and lactalbumin.

The total nitrogen content of the "membrane" proteins.—All the "mem-

brane'' proteins, both from natural and artificial creams, have a low nitrogen value, much lower than observed for pure proteins. The cause for this low nitrogen content is not known. Attempts to detect carbohydrate or sterol as the prosthetic group of the natural ''membrane'' protein have not proved successful; also the low nitrogen is not explained by a high mineral content.

DISCUSSION

Those substances in milk plasma which lower the tension of a butter-fat-water interface would be expected to concentrate at the surface of the fat globules. However, in milk it is evident that the fat globules are wholly or partially surrounded by a special group of substances whose origin has not yet been explained and whose presence may or may not be due wholly to their colloidal properties. The surface active substances occurring in major concentration in milk plasma evidently constitute the outer layers of the fat globule surfaces if indeed they are normally concentrated there at all. For when cream is washed by dilution with water the surface active substances of milk plasma are readily removed leaving essentially only the special, probably highly oriented material as the natural ''membrane'' layer around the fat globules.

This special ''membrane'' material has been shown in this study to be composed of protein, phospholipides, and ether extractable non-phospholipides. This is in agreement with the earlier work of Palmer and Samuelson (5) and Palmer and Wiese (2). The significance of the ether-extractable non-phospholipide material which composes over 50 per cent of the ''membrane'' can not be stated at the present time. It is also found in the fat globule ''membranes'' of the artificial creams. Perhaps the oriented molecules of ''membrane'' material are partly in the fat phase and are attached to certain of the fat molecules with almost chemical affinity; when the fat globules coalesce during the churning process to become the continuous phase the ''membrane'' molecules possibly continue to hold onto this fat which can only be removed by fat solvents.

It was found in this study that the ''membrane'' composition is variable from one natural cream to another in respect to the protein and phospholipide content, and also in respect to the Van Slyke nitrogen distribution of the protein fraction although the latter variation is only slightly significant. The cause of these variations remains to be determined. The mechanical methods employed are no doubt responsible in part but the possibility of certain variations being of biological origin, *i.e.*, natural variations, cannot be overlooked.

Repeated washing with water slowly wears away the ''membrane'' around the fat globules in natural cream, but it takes more than twelve washings before the emulsion begins to break, and even after twenty-four

washings the cream remaining still contains fat globules with their "membrane" intact.

Washing, water-washed cream, with whey in some way affects the lipide phosphorus fraction so that this can be washed away by further water washing much more readily than when water alone is used. This is one of the most surprising results of the present study and shows that the lipide fraction cannot be regarded as a chemical component of the "membrane" protein molecules.

The "membrane" materials isolated from artificial creams made by emulsifying butter-fat in either whey, skim milk or buttermilk were distinctly different from those isolated from natural creams. In the first place these artificial creams could not be washed as extensively as could the natural creams. Furthermore the "membrane" around the fat globules in artificial creams contained little or no phospholipides, the largest amount occurring in the "membrane" from the buttermilk cream. The proteins from the fat globule "membranes" of the artificial creams were found to be distinctly different from the natural "membrane" proteins in respect to Van Slyke nitrogen distribution and phosphorus analyses. Even the "membrane" isolated from buttermilk creams was not identical with the natural "membrane" materials, although buttermilk is known to contain these materials. This seems contrary to the experience of Wiese and Palmer (6) who found that artificial buttermilk cream churned normally. The explanation may lie in the fact that the unwashed buttermilk cream is as rich in phospholipides as many samples of washed natural cream (See Table 1). It is possible, therefore, that a small but significant part of the "membrane" in this case is the natural "membrane" material occurring in the buttermilk, the amount being sufficient to account for its normal churning behavior.

These results show that the recent work of North and Sommer (7) may be criticized because they assume that by streaming diluted skim milk through a butter-fat capillary they are dealing with the same "membrane" that is found around natural fat globules in milk. The emulsions of butter-fat in skim milk do not produce typical "membrane" material around the fat globules as evidenced by the chemical analyses reported in this paper and the other abnormal behavior of artificial skim milk creams.

The Van Slyke nitrogen distribution of the fat globule "membrane" proteins of natural cream resembles more the nitrogen distribution of the "membrane" proteins isolated by Hattori (1) and Wiese and Palmer (2) than that of the "membrane" proteins isolated from artificial creams or that of the ordinary milk proteins. The "membrane" proteins from the artificial creams resemble mixtures of known milk proteins in regard to their Van Slyke nitrogen distributions.

This dissimilarity between the fat globule "membrane" materials of artificial creams and the natural "membrane" material raises the question

of the origin of the natural fat globule "membrane." It appears possible that the "membrane" may be formed before the fat globules become a part of the milk or the fat globules may be secreted before the milk plasma is completely formed in which case the "membrane" materials could be considered, in part at least, as precursors of plasma materials.

The nitrogen content of fat globule "membrane" proteins of both the artificial and natural creams is lower than that observed for known proteins. We have no explanation for these low total nitrogen values at the present time, but we do know they are not due to cholesterol, carbohydrate or to the presence of large amounts of inorganic materials since analyses have been made for these substances.

Chrzaszcz and Goralowna (14) have shown that diastase, reductase and catalase are present in higher concentration in cream than in milk; Wieland and Macrae (15) have shown this to be true also of dehydrogenases; Tayama (16) has demonstrated that zanthine oxidase is present on washed fat globules; and Kay and Graham (10) have shown that phosphatase is closely associated with the fat globules in milk. Our study has demonstrated conclusively that phosphatase is a definite constituent of the natural fat globule "membrane." The various enzymes mentioned and others for which tests have not been made possibly play an important part in the keeping qualities of the fat-rich fractions of milk. This is suggested by the fact that in the preservation of the samples from the cream washing experiments certain samples showed a deterioration of the phospholipide fraction, which was probably due to enzyme action, possibly a lecithinase.

CONCLUSIONS

1. The percentage of protein and phospholipides in the fat globule "membrane" from various samples of cream is not constant but is essentially constant for any one sample of cream after the fourth washing with distilled water, at least through the tenth washing.

2. The adsorption "membrane" found on butter-fat globules emulsified in sweet rennet-whey, skim milk, or buttermilk does not have the same composition as the natural fat globule "membrane" of milk in its percentage and proportion of protein and phospholipides.

3. The Van Slyke nitrogen distribution of the fat globules "membrane" proteins from artificial creams prepared by emulsifying butter-fat in whey, skim milk or buttermilk, is not the same as that of the natural fat globule "membrane" proteins.

4. A large part of the phosphatase activity of natural cream is in the fat globule "membrane" material and is not removed by water washing.

5. In the synthesis of milk the natural fat globule "membrane" is not derived from the milk plasma.

6. The fat globule "membrane" proteins of natural cream and of artificial creams made by emulsifying butter-fat in skim milk, buttermilk or whey appear to contain a prosthetic group, so far not identified, which causes the nitrogen content to be abnormally low as compared with other known proteins of similar complexity.

TABLE 1

The effect of washing cream on its lipide phosphorus, total phosphorus and protein content

CREAM	PHOSPHORUS PER 100 GRAMS FAT				PROTEIN PER 100 GRAMS FAT
	Lipide[1] P			Total P	
	1	2	3		
	mgms.	*mgms.*	*mgms.*	*mgms.*	*grams*
Natural creams (washed with water)					
Cream 1					
Original cream	17.3			188.3	11.4
After first washing	12.9			39.7	1.66
After second washing	9.3			18.6	0.73
After third washing	8.7			15.3	0.61
After fourth washing	7.2			13.8	0.59
Cream 2					
After fourth washing	8.3			11.8	0.45
After eighth washing	10.6		5.8	12.2	0.46
After tenth washing	8.5			11.2	0.45
Cream 5					
Original cream	15.6			128.8	
After fourth washing	13.6	10.2	8.6	18.2	0.69
After eighth washing	11.1		8.7	16.4	0.61
After twelfth washing	7.1	7.3		16.5	0.54
After twenty-fourth washing	5.4			10.4	0.46
Cream 6					
Original cream	20.2	17.8			
After fourth washing	13.3	15.0	14.0	21.3	
After eighth washing	11.3	12.5	11.2	16.6	0.71
Cream 7					
Original cream	18.8	16.5			
After fourth washing	13.6	12.3		18.2	
After eighth washing	10.3		10.9	17.9	0.68
Cream 8[2]					
After fourth whey washing	10.3			137.2	
After first water washing[3]	7.2			26.5	
After second water washing	4.3			12.2	
After third water washing	4.1			10.3	
After fourth water washing	3.7			9.3	0.36
Artificial creams (washed with water)					
Cream 9 (whey cream)					
Original cream	3.39				
After third washing	0.66			2.8	0.57
Cream 10 (skim milk cream)					
Original cream	0.68			189.0	
After fourth washing	0.14			5.5	0.65
Cream 11 (buttermilk cream)					
Original cream	7.63			191.6	
After first washing	1.50			37.0	
After third washing	1.20			25.8	

[1] Analyses 1, 2 and 3 were made on the same sample at about week intervals.
[2] Washed with water 4 times, whey 4 times, then water 4 times.
[3] Following the whey washings.

TABLE 2

Phosphatase activity of cream washed with water and of the buttermilk from the cream washed six times

	PHOSPHATASE UNITS[1] IN CREAMS	PHOSPHATASE UNITS[1] PER GRAM OF FAT
Cream 4		
Original cream	3.00 per gram	6.84
After first washing	1.99 " "	5.73
After fourth washing	1.69 " "	4.94
After sixth washing	1.52 " "	4.22
	1.43 per cc. 4.18 per cc.	3.41

[1] Kay and Graham units (10).

TABLE 3

Composition of the fat globule "membrane" isolated from natural and artificial creams

	PROTEIN	ALCOHOL-ETHER EXTRACT		
		Phospholipide[1]	Non-phospholipide	Total
Normal creams	*per cent*	*per cent*	*per cent*	*per cent*
Cream 2				
Washed four times with water	43.96	17.53	38.52	56.04
Washed eight times with water	40.12	19.46	40.42	59.88
Washed ten times with water	39.13	21.96	38.91	60.87
Cream 3				
Washed four times with water	29.13	26.00	44.87	70.87
Cream 4				
Washed six times with water	21.98	.....	.....	78.02
Cream 6				
Washed eight times with water	36.30	13.00	50.70	63.70
Cream 7				
Washed eight times with water	33.29	22.51	43.80	66.71
Cream 8				
Washed four times with water, four with whey, and four with water	34.76	.....	.....	65.24
Artificial creams				
Cream 9 (whey cream)				
Washed three times with water	54.43	.....	.....	45.57
Cream 10 (skim milk cream)				
Washed four times with water	44.66	.....	.....	55.34
Cream 11 (buttermilk cream)				
Washed three times with water	55.25	.....	.....	44.75
Cream 12 (buttermilk cream)				
Washed three times with water	52.25	.....	.....	47.75

[1] Calculated from the percentage total phosphorus in alcohol and ether extract using factor 3.85% P in phospholipide.

TABLE 4

Average[1] analyses of fat globule "membrane" proteins

	CREAM 2[2]	CREAM 3[3]	CREAM 4[4]	CREAM 8[5]	CREAM 9[6]	CREAM 10[7]	CREAM 11[8]	CREAM 12[9]	PREVIOUS ANALYSES[10]
Van Slyke Nitrogen Distribution per cent									
Ammonia-N	11.94	10.16	11.58	11.68	13.19	13.22	11.31	11.98	6.54
Humin-N	4.69	3.73	4.21	4.58	3.78	3.19	2.74	2.48	3.86
Arginine-N	13.59	10.77	12.16	11.27	10.06	9.64	7.36	7.43	15.82
Cystine-N	0.27	0.48	0.675	0.70	0.73	0.46	0.37	0.49	0.72
Histidine-N	1.00	5.64	3.63	3.67	3.10	3.60	2.41	5.02	1.69
Lysine-N	11.36	8.60	10.27	8.53	12.19	11.68	13.08	11.53	11.49
Basic NH_2-N	15.42	13.64	15.20	13.27	16.48	15.75	16.10	15.55	16.73
Total basic-N	26.23	25.49	26.74	24.18	26.09	25.38	23.23	24.47	29.71
Non basic NH_2-N	55.69	57.51	55.79	57.83	54.20	53.96	57.73	55.56	56.50
Non basic non NH_2N	4.20	3.84	3.68	3.20	3.99	4.57	5.51	7.08	3.51
Total non basic-N	59.89	61.35	59.47	61.03	58.19	58.63	63.24	62.64	60.01
Total recovery	102.76	100.73	102.00	101.48	101.26	100.43	100.53	101.58	100.12
Total N	13.07	13.48	13.45	12.74	12.35	13.31	13.44	13.75	12.22
Phosphorus	0.492	0.489	0.388	0.446	0.350	0.877	1.290	1.350	0.33
Calcium		0.484	0.244						
Ash		2.83	2.00						

[1] Average of two analyses agreeing within the limits permitted for these determinations (Cavett (1932)).
[2] Natural cream washed ten times with water.
[3] Natural cream washed four times with water.
[4] Natural cream washed six times with water.
[5] Natural cream washed four times with water, four times with whey and then four times with water.
[6] Artificial whey cream washed three times with water.
[7] Artificial skim milk cream washed four times with water.
[8] Artificial buttermilk cream washed three times with water.
[9] Artificial buttermilk cream washed three times with water.
[10] Average of analyses made by Wiese and Palmer (3).

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American Dairy Science Association Announcements

ANNUAL MEETING, JUNE 16–19, 1936

The annual meeting of the American Dairy Science Association will be held at the Pennsylvania State College June 16–19, 1936. The Program Committee will welcome suggestions from the membership concerning new features or changes from the type of program that we have followed in past meetings. Suggestions may be sent directly to S. I. Bechdel, chairman of the Program Committee, or to any of the general officers of the Association or to the officers of the Sections.

NEW OFFICERS

Results of the election of officers are not generally mailed to members but the directory page as carried in the Journal is corrected as rapidly as changes are brought to the attention of the editor. It is believed that list now contains all of the newly elected officers, most of whom took office on October first.

JOURNAL INDEX

The system of indexing the Journal has been changed in the present volume to care for the increased size of the Journal.

The complete papers and abstracts of papers given at the annual meeting have been grouped together and each article appears at least three times in the subject index. The author index has been separated from the subject index. Since the editor has planned on following this same style of index and similar main index headings, comments to improve it would be appreciated.

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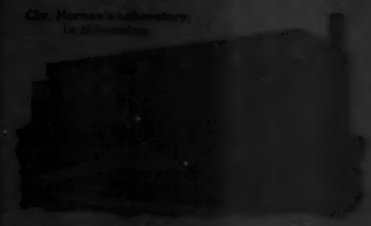

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